

ON THE WEIGHT OF THE ALBINO RAT AT BIRTH AND THE FACTORS THAT INFLUENCE IT

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In the course of an extensive series of breeding experiments with the albino rat a large amount of data has been collected regarding the body weight of these animals at different stages of their growth. The records dealing with the weight at birth are given in the present paper: those of postnatal growth will be published later.

Two sets of observations on the body weight of very young albino rats have already been recorded. In a paper published by Donaldson in 1906 the average weight of 40 young male albino rats is given as 5.4 grams, and that of 17 females is stated to be 5.2 grams. The more extensive records of Jackson ('13) give the average weight of 107 young male albino rats as 5.1 grams, and that of 109 females as 4.8 grams.

The body weights, as given above, are those of animals that were 'newborn' when weighed. The term 'newborn,' as Jackson states, covers the period in the life of the animal from the time of birth up to one day. As a rule, young rats begin suckling very soon after their birth, and not infrequently part of a litter will have suckled before the rest have been born. The weight of 'newborn' animals, therefore, is probably not the same as the birth weight in many cases. To obtain the birth weight it is necessary that the animals be weighed before they have suckled, since the amount of food consumed during the first few hours of postnatal life very appreciably increases the body weight. One can tell very easily whether or not the young rats have suckled, as the skin of the young animals is quite

transparent and if milk is present in the stomach or in the intestines it can be seen very clearly through the body wall.

In the course of this investigation 113 litters of rats were obtained at or soon after birth and weighed before any of the individuals had taken food. The same course of procedure was followed in making the records for each of the litters. The young rats were first separated according to sex by the method devised by Jackson ('12). Animals of the same sex were then weighed together to a tenth of a gram and the average weight for each individual computed; if, however, there was a very marked difference in the size of the individuals of the same sex the rats were weighed separately and the weight of each recorded. In addition to recording the number of young, the sex distribution and average body weight of the members of each litter, the exact age of the mother at the time the litter was born was noted, also her body weight after the birth of the litter and her general physical condition.

The complete series of records comprise data from five different strains of rats that are being bred in The Wistar Institute animal colony at the present time:

1. Stock albinos: Members of the general colony that supposedly represent the normal albino type as it exists at the present time.
2. Inbred albino rats: Animals, originally taken from the general stock colony, that have been closely inbred for many generations.
3. Extracted albinos: A strain of rats descended from albinos cast by F_1 hybrids of the albino and the wild Norway rat (*Mus norvegicus*).
4. Piebald rats: A strain derived from F_1 hybrids of the albino and Norway rat.
5. Extracted grays: A strain also derived from the F_1 hybrids of the albino and the Norway rat.

Table 1 gives a general summary of the birth records arranged according to the strain of rats from which the litters were obtained. The data in this table show that, regardless of strain, the

weight of the albino rat at birth is considerably less than that of newborn animals as given by Donaldson and by Jackson. As a rule the male rat at birth is somewhat heavier than the female, as is the case in many other mammals including man.

TABLE 1

Showing the birth weight data for various strains of rats

STRAIN OF RATS	NUMBER OF LITTERS	NUMBER OF INDIVIDUALS	MALES	FEMALES	TOTAL WEIGHT OF MALES IN GRAMS	TOTAL WEIGHT OF FEMALES IN GRAMS	AVERAGE WEIGHT OF MALES IN GRAMS	AVERAGE WEIGHT OF FEMALES IN GRAMS
Stock albinos	12	95	47	48	215.6	216.1	4.59	4.50
Inbred albinos	73	644	311	333	1409.5	1410.8	4.53	4.23
Extracted albinos	8	43	21	22	88.2	88.2	4.20	4.00
Piebalds	19	147	80	67	386.1	324.3	4.82	4.84
Extracted grays	1	9	4	5	22.9	27.7	5.72	5.54
Total	113	938	463	475				

On comparing the data for the various strains of rats it is found that stock albinos, both males and females, weigh slightly more at birth than do the inbred rats. The differences between them are not sufficiently great to have much significance, especially as the number of stock litters that was weighed was relatively small. Extracted albinos weigh considerably less at birth than either the stock or inbred rats. This fact is not surprising considering that these animals grow much less rapidly than stock or inbred albinos and that many of them, particularly the females, fail to attain the average adult size of stock animals. Piebald rats, both males and females, have a birth weight that is greater than that of any of the albino strains.

The average weight of the piebald females, as given in table 1, is slightly greater than that of the males. This is probably a chance variation, since in 12 of the 19 litters that were weighed the average weight of the males exceeded that of the females. From the single litter of extracted gray rats weighed at birth

one can obtain but little idea of the birth weight for the strain, yet the records are significant in that they show an average weight for both sexes that is close to the mean between the birth weight of the wild Norway rat, which is about 6.4 grams for both sexes according to Miller ('11) and that of the albino rat.

In any litter of rats, as a rule, individuals of the same sex are practically of the same size and body weight at birth. Occasionally, however, very marked exceptions to this rule are found. In one of the litters of inbred rats the difference in the weights of the various individuals was so unusually great as to call for more than a passing notice.

The litter in question contained eleven individuals, four males and seven females. One of the males, which was the largest rat yet obtained at birth, weighed 7.5 grams; the other three males were nearly uniform in size, weighing 4.9 grams, 5.0 grams and 5.1 grams, respectively. The females in this litter also showed considerable variation in body weight. The largest of the seven females weighed 4.6 grams, the smallest weighed 2.9 grams. The latter is not the smallest birth weight for the rat that has been obtained, however, as in one case in which the mother of the litter was in the last stages of pneumonia when the litter was born, two of the three males in the litter weighed 2.6 grams each; the third male weighed 2.9 grams which was also the weight of the one female in the litter. Female rats do not seem to show as great a variation in body weight at birth as do the males. The largest female yet obtained weighed 5.9 grams at birth; the smallest weighed 2.7 grams.

It seems most probable that such marked variations in the size of the different members of a litter at birth as are shown above must be due to a difference in the age of the embryos at the time that parturition occurs, not to causes acting late in gestation. Evidence already presented (King '13) indicates that, under certain conditions, ovulation in the rat may extend over three or four days, possibly longer. Ova liberated at various intervals for several days would probably all be fertilized, as the period of heat in the rat exists for about one week (Miller '11). The body growth of the embryos is very rapid during the latter part

of gestation, and embryos that developed from the ova first set free might be expected to have a greater body weight than the embryos that developed from ova liberated late in the period of ovulation, since they would have a longer time in which to grow. Rats born at one period of parturition, however, must be very nearly the same age, as the smaller individuals show no evidence of immaturity other than in their smaller size. If there is considerable difference in the age of the embryos developing simultaneously in the uterus the more mature ones are born first, and the remaining ones are born from one to several days later when they have reached the proper stage of development (King '13).

The variations in body weight found among rats at birth are seemingly greater than those in 'newborn' animals. The largest male in Donaldson's series weighed 6.5 grams, the smallest weighed 4.3 grams: corresponding figures for the females give 6.2 grams as the heaviest weight and 4.2 grams as the lightest weight. In Jackson's series the weights of the males range from 3.4 grams to 6.6 grams and those of the females from 3.5 grams to 6.3 grams.

There is a possible explanation for the narrow range of variation in the weights of 'newborn' rats besides the obvious one that the series of animals weighed was too small to contain the extreme variates in body weight. Individuals that are very small at birth may increase in body weight and in size more rapidly during the first few hours of postnatal life than the members of the litter that have a heavier birth weight. This would tend to equalize the size of the individuals and so give all of them approximately the same chances of obtaining food. The early growth changes in the rat have not been studied sufficiently as yet to give evidence on this point.

In analyzing the data collected in connection with the birth weights with a view of ascertaining, if possible, some of the factors that help to determine the weight of the rat at birth, it has been considered advisable to make use only of the records for the 85 litters of stock and of inbred albino rats. The average weight of the young in the piebald and in the extracted gray litters is so much greater than that of the albinos that the effects

of these records on the general averages, when taken in small groups, would be altogether disproportional to the number of individuals involved. The birth weights for the extracted albinos, on the other hand, fall so far below those for the other albinos that they seem properly to belong in a class by themselves. It does not seem worth while to analyze the data for these strains otherwise than in the manner shown in table 1. The records are as complete as for the stock and inbred albinos, however, and are filed at The Wistar Institute.

THE EFFECTS OF THE AGE OF THE MOTHER ON THE WEIGHT OF HER YOUNG AT BIRTH

Slonaker ('12) states that the age of the mother affects not only the number of young rats in a litter but also their weight at birth, young mothers being less prolific than older ones. He makes no mention, however, of the extent of the data on which this conclusion is based.

Under the conditions existing in The Wistar Institute animal colony the female albino rat usually has her first litter when she is about three months old, and she is capable of bearing young until she is about fifteen months old. In order to study the effects of the age of the mother on the weight of her young at birth the reproductive period in the life of the albino female has been arbitrarily divided into the four following periods:

1. From 90 to 120 days: This is the age when young females are growing very rapidly and the time when the great majority of them cast their first litters.

2. From 120 to 180 days: During this time the female reaches the end of the rapidly growing period and becomes fully mature.

3. From 180 to 300 days: The female is at the height of her reproductive powers during this period and has attained full growth.

4. From 300 to 450 days: In this period there is a dying out of the reproductive power and little, if any, growth.

Table 2 shows the data for the 85 litters of stock and of inbred albino rats arranged in four groups according to the age of the

mother at the time that the litter was born. The data, as arranged in this table, do not show the gradual increase in the body weights of the young from the first to the fourth group that one would expect to find if the age of the mother is the dominant factor in determining the birth weight of her young. If, however, we compare the average birth weight of the rats in litters cast by females during the first reproductive period with that of the individuals belonging to litters born when the reproductive power of the mother is waning, it is found that the average body weights in the first group are considerably less than those in the last group. The difference between them, amounting to 0.3

TABLE 2

Showing the birth weight data for 85 litters of stock and inbred albino rats arranged according to the age of the mothers at the time that litters were cast

AGE OF MOTHER	NUMBER OF LITTERS	NUMBER OF INDIVIDUALS	MALES	FEMALES	TOTAL WEIGHT OF MALES IN GRAMS	TOTAL WEIGHT OF FEMALES IN GRAMS	AVERAGE WEIGHT OF MALES IN GRAMS	AVERAGE WEIGHT OF FEMALES IN GRAMS
(1) From 90 to 120 days	27	232	112	120	494.9	494.6	4.41	4.12
(2) From 120 to 180 days	36	326	155	171	712.1	736.9	4.59	4.30
(3) From 180 to 300 days	17	143	70	73	318.5	321.5	4.55	4.40
(4) From 300 to 450 days	5	38	21	17	99.2	73.3	4.71	4.31

gram in the case of the males and 0.2 gram in the case of the females is sufficiently great, I think, to warrant the conclusion that the weight of a litter at birth depends, to a certain extent, on the age of the mother. During the first reproductive period young females are growing very rapidly both in body size and in body length and presumably, therefore, the growth processes consume a considerable part of all available energy. Litters cast by females at this time contain, as a rule, few individuals and these are of relatively small size. In older females growth has practically stopped and more energy can be used for the production of larger litters containing individuals that have a heavier weight at birth.

THE INFLUENCE OF THE BODY WEIGHT OF THE MOTHER ON THE BIRTH WEIGHT OF HER YOUNG

Under normal conditions body weight and age are closely correlated in the rat, as Donaldson's investigations have shown. Body weight, however, being easily affected by changes in environment or in nutrition is, to a certain extent, independent of the age factor and indicates very clearly the physical condition of an animal. Rats that are heavy for their length and age are usually in excellent health; those that are light in weight are generally ill, as certain diseases—for instance, 'pneumonia'—may be shown by a rapid drop in the weight of the animal before any other symptoms of illness are manifested. The body weight of a female, as indicating her general physical condition irrespective of age, may possibly, therefore, be a factor that would tend to influence the birth weight of her young.

As age is the factor that so largely determines body weight in the rat, it has seemed advisable to group the records for the body weights of the females according to age. To do this it is necessary to know the body weights that are normal for various ages. Table 3, compiled from an extensive series of unpublished data collected in the course of my breeding experiments, gives the normal weight of stock and of inbred albino females that correspond with the age groups used as the basis of analysis in the previous section. Inbred albino females are slightly heavier

TABLE 3

Showing the body weight of stock and of inbred albino rats normal for different age groups

AGE OF FEMALES IN DAYS	NORMAL WEIGHT OF STOCK ALBINO FEMALES	NORMAL WEIGHT OF INBRED ALBINO FEMALES
90 to 120 days	148 to 173 grams	156 to 175 grams
120 to 180 days	173 to 195 grams	175 to 199 grams
180 to 300 days	195 to 219 grams	199 to 221 grams
300 to 450 days	219 + grams	221 + grams

for a given age than are stock albino females, as is shown in table 3. Since the great majority of the birth weights recorded are of litters belonging to the inbred strain, the body weights

of the inbred females have been used as the basis for the grouping of the data in the present instance.

Table 4 gives the various birth weight records arranged according to the body weight of the mothers at the time that parturition occurred. If the body weight of all the females had been normal for the age at which their litters were cast, table 4 would be practically a duplicate of table 2, where the data are arranged according to the age of the mothers. A comparison of the two tables shows, however, a very different distribution

TABLE 4

Showing the birth weight data for stock and inbred albino rats arranged according to the body weight of the mothers at the time that the litters were cast

BODY WEIGHT OF FEMALE	NUMBER OF LITTERS	NUMBER OF INDIVIDUALS	MALES	FEMALES	TOTAL WEIGHT OF MALES IN GRAMS	TOTAL WEIGHT OF FEMALES IN GRAMS	AVERAGE WEIGHT OF MALES IN GRAMS	AVERAGE WEIGHT OF FEMALES IN GRAMS
To 175 grams	27	209	98	111	423.8	440.7	4.46	3.86
175 to 200 grams	23	222	108	114	491.3	506.8	4.55	4.47
200 to 220 grams	25	233	111	122	508.7	524.7	4.58	4.30
220 + grams	10	75	41	34	201.7	158.0	4.91	4.64

of litters in the corresponding groups. The first group in each table happens to contain the same number of litters, but in table 4 this group comprises 9 litters from females that were over 120 days of age when parturition occurred. These females had fallen below the normal weight for their age and were, presumably, not in especially good physical condition. The second group in table 4 contains 6 litters from females younger than 120 days, and 4 litters from females that had passed 180 days of age when the litters were cast. In this group, therefore, 10 of the 23 litters belonged to females that did not have a normal body weight. Eighteen of the 25 litters belonging to the third group of table 4 were cast by females younger than 180 days of age and two of them by females older than 300 days. In the last group only three of the 10 litters came from females that had a body weight normal for the age of which the litters were cast.

According to the records as given in table 4, the average weight of young rats at birth increases directly as the body weights of the mothers increase. When the body weights of the females are below 175 grams the average weight of the young males in the litters is 4.46 grams. This average rises to 4.91 grams for the males in the litters cast by females weighing 220 grams or more. Records for the female young do not show quite such uniformity as in the case of the males, since the weights of the individuals belonging to the third group are less than those of the individuals in the second group. The weight of the females in the fourth group, however, is greater by 0.78 grams than that of the females belonging to the first group. This difference is considerably greater than that shown by the males in the two groups.

That these results depend to a considerable extent on the age of the mothers of the litters there can be little doubt, since body weight is closely associated with age and in the normal animals the range of variation is not very great.

If, however, we disregard the age factor and take the body weights of the mothers as indicative of the physical fitness of the animals, it is evident that the heavier females, being in excellent condition, tend to produce young that are larger at birth than the young cast by females that are relatively light in weight and probably, therefore, not in very good condition.

From this analysis of the data it follows that it is not the body weight of the female in itself, but the factors on which the weight largely depend, i.e., age and physical condition, that have a pronounced influence on the birth weight of the young.

THE INFLUENCE OF THE LITTER SIZE ON THE WEIGHT OF THE YOUNG AT BIRTH

The size of a litter of albino rats depends, seemingly, on a number of different factors, one of which is undoubtedly the age of the mother. Very young females and those that have passed their prime have smaller litters, as a rule, than females at the height of their reproductive powers. The physical condition of the mother is also a factor that apparently affects the litter size,

as females in poor condition rarely have a large litter, and if the number of young exceeds the average for the species several of them are usually stillborn. Litter size therefore is another factor so inseparably linked with the age and physical condition of the mother that its influence on the birth weight of the young must be considered in connection with the other factors involved.

Unpublished data for over 1000 litters of stock albino rats show that the average litter contains seven young. The size of the litter varies greatly in different cases, the range being from one to thirteen.

TABLE 5

Showing the birth weight data, for stock and inbred albino rats, arranged according to the size of the litter. G, litters cast by females in good physical condition; P, litters cast by females in poor condition

SIZE OF LITTER	NUMBER OF LITTERS	NUMBER OF INDIVIDUALS	MALES	FEMALES	TOTAL WEIGHT OF MALES IN GRAMS	TOTAL WEIGHT OF FEMALES IN GRAMS	AVERAGE WEIGHT OF MALES IN GRAMS	AVERAGE WEIGHT OF FEMALES IN GRAMS
5 or less	4(G) 5(P)	18 24	9 10	9 14	45.1 37.3	39.6 50.8	5.01 3.73	4.33 3.62
6 to 8	30	217	111	106	512.6	464.7	4.61	4.38
9 or more	46	480	228	252	1030.0	1075.7	4.51	4.26

In order to analyze the birth records on the basis of litter size, the litters have been arbitrarily divided into three groups: small litters, containing five or less young; medium sized litters, containing six to eight individuals; large litters with nine or more young.

The arrangement of the data according to the above classification is shown in table 5. The data, as shown in this table, seem directly opposed to the generally accepted view that animals belonging to small litters weigh more at birth than those belonging to large litters, since the average weight of both males and females is considerably less for the small litters than for the very large ones. The record cards show, however, that five of the nine small litters were cast by females that were in poor physical condition. In these five litters the average weight

of the males was 3.73 grams and that of the females was 3.62 grams; in the four litters from females apparently in good condition the average weight of the males was 5.01 grams and that of the females was 4.40 grams. Only seven of the 85 litters weighed were cast by females in poor condition, and five of them, as shown above, belong in the small litter group. The other two were litters of medium size, and if their records were omitted from table 5 the average birth weight of both males and females in this group would be raised slightly.

It seems justifiable, therefore, to disregard the data for the five small litters cast by females in ill health and to take the records for the four litters cast by females in good condition as representing the birth weights for the small litter group. If this be done, the data in table 5 show that the average weight of the young in small litters greatly exceeds that for the individuals in large litters: individuals in medium sized litters weigh close to the mean between the weights of the animals in the small and in the large litters. This rule holds true for animals of both sexes, and seems to indicate that the birth weight of young rats depends to a considerable extent on the size of the litter, irrespective of the other factors that may be involved.

Additional evidence that the size of the litter influences the birth weight of the young is furnished by the records for the largest litter of albino rats as yet obtained. This litter, which belonged to the inbred strain, contained 17 individuals, 10 males and 7 females. The young rats in this litter were several hours old when first examined and they had all suckled, so that it was not possible to obtain their birth weights. The average weight of the 17 individuals at the time they were found was 2.7 grams. At birth, therefore, these rats probably weighed not more than 2.5 grams each. The smallest birth weight for the rat that has as yet been taken is 2.6 grams. In this litter, therefore, the very small size of the individuals was undoubtedly due to the exceptional size of the litter, as the mother of the litter was large for her age and seemingly in the best of condition when the litter was born.

In a study of the weight of guinea-pig Minot ('91) found that the size of the pigs at birth depends to a considerable degree on the number of young in a litter; the larger the litter the smaller the pigs at birth. According to Minot, the litter size influences the birth weight by changing the length of the gestation period.

When the litter is large the gestation period is shortened and therefore the young pigs do not have as long a time in which to grow as is the case when the litter is small and consequently the gestation period longer. The birth weight of guinea-pigs, therefore, does not depend, according to Minot, on "the ratio of food supply and demand" but on the length of gestation.

In the rat, as in the guinea-pig, the length of the gestation period varies considerably in different cases. Normally it is from 21 to 23 days, but in lactating females it may be extended to 34 days (King '13). As far as I am aware, no attempt has been made as yet to ascertain the relation between the number of young in a litter and the duration of gestation in the rat. When a lactating female becomes pregnant the number of young in the second litter is certainly a factor of considerable importance in determining the length of the gestation period, for gestation is prolonged from one to thirteen days if the number of young carried is very large. How the birth weight of the young is affected by this prolongation of the gestation period remains to be determined. Growth is so rapid during the latter part of gestation that the extension of the period for even one day might be expected to materially increase the size of the embryos unless other factors tend to check growth at a certain stage of development.

THE RELATION OF THE LITTER SERIES TO THE WEIGHT OF THE YOUNG AT BIRTH

To arrange the records for the birth weights according to the position of the litters in the litter series would seem to be merely another way of testing the effect of the age of the mother on the weight of her young at birth, since it is impossible to eliminate the factor of age in carrying out such a plan. Female rats show very great individual differences, however, in regard to the

time when their litters are produced. Some rats begin breeding before they are three months old; others do not have their first litter until they are four or six months old. Certain females will cast a litter every month for several succeeding months; others never have a litter oftener than every two months and not infrequently three or four months will intervene between litters even when the females are apparently in excellent condition.

The plan of the breeding experiments at present under way requires that every breeding female shall have four litters. Some females have the required number of litters by the time they are six months old; others not until they are a year or more old. While, therefore, the range of variation in the age of the mothers is comparatively slight for the first and for the second pregnancies, it may be extended over several months before the third and the fourth litters are produced.

The litter series must always be, in a sense, an age series, yet the individual variations in the frequency of the litter production are sufficiently great, I think, to make it worth while to study the birth weight records from the point of view of the number of the pregnancy.

Table 6 shows the records for birth weights arranged according to the litter series. An analysis of the litters from several hundred females has shown that the first of a rat's four litters

TABLE 6

Showing the birth weight data for stock and inbred albino rats arranged according to the position of the litters in the litter series

LITTER SERIES	NUMBER OF LITTERS	AVERAGE NUMBER OF YOUNG IN LITTER	NUMBER OF INDIVIDUALS	MALES	FEMALES	TOTAL WEIGHT OF MALES IN GRAMS	TOTAL WEIGHT OF FEMALES IN GRAMS	AVERAGE WEIGHT OF MALES IN GRAMS	AVERAGE WEIGHT OF FEMALES IN GRAMS
1.....	22	8.5	187	91	96	400.0	390.2	4.39	4.06
2.....	21	8.9	188	82	106	370.8	452.7	4.52	4.27
3.....	25	9.4	236	125	111	576.6	492.5	4.61	4.43
4.....	17	7.5	128	60	68	278.6	296.5	4.64	4.36

is usually the smallest, the second and the third the largest, and the fourth a little larger than the first. This rule, however, does not happen to apply in the case of the litter series shown in table 6. Not only is the average number of young in the first group considerably above that which is normal for the species, but it greatly exceeds that for the fourth group; the second and third groups are fairly normal in size. Litter size is, in all probability, a factor that in the present instance does not materially affect the birth weights of the young rats, since the average size of the litters varies but little for the four groups.

Table 6 shows that the birth weights of the males increase with the ascending scale of the litter series; for the females there is a slight irregularity in the figures for the third and fourth groups, but the average weight of the young females in the fourth group is 0.3 grams greater than that in the first group. A comparison of these birth weights with those given in table 2, where the age of the mother formed the basis of the classification of the data, shows that the figures for corresponding groups are much the same, the deviation in no case being greater than 0.07 grams. No other tables given show such close agreement, and, therefore, it appears that the position of a litter in the litter series influences the birth weight of the young chiefly because it so closely involves the factor of age.

THE EFFECTS OF THE PHYSICAL CONDITION OF THE MOTHER ON THE WEIGHT OF HER YOUNG AT BIRTH

One accustomed to the care and handling of rats can quickly tell from the general appearance and weight of an animal whether or not it is in good physical condition. A hunched back, labored breathing, dark red eyes and a relatively light weight indicate internal disorders from which the rat rarely, if ever, recovers. On the other hand, a heavy weight for body size and pronounced vigor in action shows that the animal is in excellent health.

Seven of the 85 females whose litters were weighed were noted as in exceptionally good condition when their litters were born, and seven others showed unmistakable signs of ill health. Records for the weights of the litters from these 14 females are given in

table 7. In the litters from females that were in excellent condition when their litters were born the average birth weight of the young for both sexes is much greater than the normal, and it exceeds the average weights of the young in the litters cast by females that were in ill health by over 1.1 grams, as is shown in table 7. This result indicates that the physical condition of the mother, irrespective of her age, is a very important factor in determining the weight of her young at birth, probably through its action on the nutritive conditions to which the embryos are subjected.

TABLE 7

Showing the birth weight data for 14 litters of stock and of inbred albino rats arranged according to the physical conditions of the mothers at the time that the litters were cast.

CONDITION OF MOTHER	NUMBER OF LITTERS	NUMBER OF INDIVIDUALS	MALES	FEMALES	TOTAL WEIGHT OF MALES IN GRAMS	TOTAL WEIGHT OF FEMALES IN GRAMS	AVERAGE WEIGHT OF MALES IN GRAMS	AVERAGE WEIGHT OF FEMALES IN GRAMS
Excellent	7	66	37	29	183.2	136.6	4.95	4.71
Poor	7	39	14	25	53.4	89.5	3.81	3.58

Another way of studying the same problem is to compare the physical condition of the mothers of the litters in which the individuals were unusually heavy at birth with the condition of the females that cast litters having a very light weight. Data for such a comparison are given in table 8, where the body weights of the mothers of the litters are taken as indicative of the physical condition of the animals. There was a total of 13 litters in which the average weight of the young of both sexes was 5 grams or over at birth. The records show that in ten cases the mothers of the litters weigh considerably more than the amount normal for their age; each of the remaining females had a body weight that corresponded exactly with her age. All 13 litters, therefore, were cast by females that were in very good physical condition as far as one could judge from the general appearance and weight of the animals.

TABLE 8

Showing the body weights of the mothers of those litters in which the birth weights of the young were above or below the normal weight

AVERAGE WEIGHT OF YOUNG IN GRAMS	NUMBER OF LITTERS	BODY WEIGHT OF MOTHERS
5 or more.....	{ 10 3	above normal normal
4 or less.....	{ 9 9	below normal normal

No definite conclusions can be drawn from the records of the 18 litters in which the young had a birth weight of 4 grams or less since the results are, in many cases, complicated by the factor of litter size. In 9 cases the body weight of the mother of the litter was below the weight normal for the age indicated; in the remaining cases the weights of the females were normal for their age, but all of the litters were very large (containing from 9 to 14 young) so litter size was doubtless a factor that had lowered the weight of the young to a certain extent. As far as the evidence from this set of records goes it seems to indicate that, in some cases at least, the small size of rats at birth is due to the poor physical condition of the mother which prevents the proper nourishment of the young and so inhibits their growth.

SUMMARY

1. Data for 113 litters show that the body weight of the young at birth differs considerably in various strains of rats. Stock and inbred albino rats weigh about the same at birth; the average weight of the males being 4.54 grams and that of the females 4.27 grams in the 85 litters that were weighed. Extracted albinos have a very low birth weight; while piebalds and extracted grays weigh much more at birth than do any of the albino strains (table 1).

2. The male rat at birth usually weighs about 0.2 grams more than does the female.

3. There is a wide range of variation in the birth weights of albino rats. The weights for the males range from 2.6 grams

to 7.5 grams; those for the females vary between 2.7 grams and 5.9 grams.

4. In any litter, as a rule, individuals of the same sex are practically of the same size and body weight. Marked exceptions to this rule are probably due to a slight difference in the age of the embryos at the time that parturition occurs.

5. The body weight of rats at birth depends upon a number of different factors that are more or less closely related:

(a) The age of the mother. Individuals in litters cast by older females weigh more at birth, as a rule, than do the individuals in litters belonging to very young females (table 2).

(b) The physical condition of the mother. Rats in good physical condition bear young with a birth weight considerably above that of the young cast by females in poor condition (tables 7, 8).

(c) The body weight of the mother. The body weight of a female influences the birth weight of her young chiefly because it depends on the two more important factors of age and physical condition. Rats that have a very heavy body weight are older and in better physical condition than rats with a light body weight, and their litters comprise individuals with a corresponding greater weight at birth (table 4).

(d) The size of the litter. Individuals in small litters weigh more at birth than do individuals in large litters (table 5).

(e) The position of the litter in the litter series. The birth weight of young rats increases directly with the ascending scale of the litter series. But, as the litter series is always an age series, it is probable that the number of the pregnancy affects the birth weight only because it involves the factor of age (table 6).

(f) The length of the gestation period. The evidence regarding the influence of this factor is slight as yet. It is very probable that the prolongation of the gestation period for even one day materially increases the weight of the young at birth.

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OBSERVATIONS ON THE ORIGIN OF THE MAST LEUCOCYTES OF THE ADULT RABBIT

PRELIMINARY NOTE

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The investigations of Maximow have shown that in mammals the connective tissue mast cells are very different from the mast cells of the blood. Maximow and Weidenreich believe that the only feature which the two types of cells have in common is the presence of basophilic granules in the cytoplasm, which stain metachromatically with basic aniline dyes. The two types of cells, however, represent independent lines of leucocyte differentiation and development, with their own peculiar nuclei and granules.

Maximow ('06) found histogenous mast cells in all the mammals he investigated, even in the rabbit where most investigators have failed. He calls attention to the fact, that where there are relatively few histogenous mast cells, the deficiency is made up by increased numbers of haematogenous mast cells, and vice versa. That such a close compensatory relationship exists between the two types of cells is shown very well in the adult rabbit, there being comparatively few histogenous mast cells, but numerous mast leucocytes.

Within the past few years the origin of the haematogenous mast leucocyte has been the subject of considerable haematological investigation. The earlier investigators, including Ehrlich, assumed that mast leucocytes were represented in the bone-marrow by certain characteristic myelocytes and evolved like the other granular cells. Weidenreich, however, has recently shown that this is not the case with the human mast leucocyte. He believes that human mast leucocytes are formed from de-

generating lymphocytes within the circulation. He derives the mast granules from the fragmenting nucleus and not from the protoplasm of the degenerating cell. Various other investigators in working on the mast leucocytes of the rabbit have come to similar conclusions with reference to the origin of these cells within the blood stream.

In 1909 Pröscher concluded from his observations that the mast leucocytes of the blood of the rabbit are merely lymphoid cells of various types whose 'spongioplasm' has undergone a special form of mucoid degeneration, which results in the formation of granules which are closely related to mucin. Mast leucocytes of the rabbit are, therefore, not true granulocytes and are not derived from myelocytes of the bone-marrow.¹

Pappenheim's students Benacchio ('11), Kardos ('11), and St. Szécsi ('12) came to similar conclusions with reference to the mast leucocytes of the guinea-pig and the rabbit. They could find no mast myelocytes in the marrow of either of these animals; they therefore concluded that the mast leucocytes are not true granulocytes. They believe that the mast leucocytes of the guinea-pig are merely eosinophil leucocytes whose granules have remained in an unripe basophilic condition. They claim that they can find all the intermediate stages between these so-called mast leucocytes and the ripe eosinophil leucocytes whose granules have an acid staining reaction. Benacchio concluded that all of the myelocytes with basophilic granules in the marrow of the guinea-pig and rabbit were either unripe eosinophiles or special cells. In other words, he believed that all of the granulocytes with basophilic granules were destined to differentiate either into eosinophiles or into special cells, and that mast cells are not present in the marrow of these animals.²

¹ Pappenheim came to similar conclusions. His views, however, are based largely upon the work of Pröscher.

² Pappenheim and St. Szécsi also believe that mast leucocytes are not represented in the marrow of the rabbit. "Die sog. Blutmastleukozyten stammen natürlich aus dem Knochenmark, aber z. T. sind sie keine eigentlichen Mastzellen, sondern nur unreifkörnige sonstige Granulocyten, deren Granula andere chromophile Reaktion hat, z. T., soweit sie eigentlichen Blutmastzellen sind, bilden sie sich aus Lymphoid-zellen wohl erst im Blut selbst oder unter pathologischer Einwirkung (Myelose)."

In a recent paper Maximow ('13) has shown that mast myelocytes are present in the bone-marrow of man, and that they are actually seen undergoing mitosis. Maximow, therefore, believes that the granules of haematogenous mast cells cannot be products of the degenerating nucleus or spongioplasm. He could never find any evidence for the degenerative processes described by Weidenreich and Pappenheim. Maximow was able to trace the differentiation of the granules and the evolution of the cells from the typical myelocytes and believes, therefore, that the haematogenous mast cells are true granular leucocytes which are equivalent to the other types of granulocytes of the blood and marrow. Maximow is also the chief exponent of the theory that the mast leucocyte of the rabbit is a true granular cell, which is in all respects equivalent to the human mast cell. He found nothing that would lead him to conclude with Benacchio, Kardos, and others that the mast leucocyte is not differentiated in the bone-marrow.

Maximow's observations on the origin of the mast leucocytes are of the greatest importance, but his observations should be confirmed by further studies, since he maintains that the mast leucocytes do not arise in the circulating blood from altered lymphocytes, but are differentiated in the marrow from certain specific, characteristic, basophilic granulocytes. Downey's³ recent studies ('13) on the mast leucocytes of the guinea-pig have resulted in the complete corroboration of Maximow's findings. He finds that the granules of mast leucocytes can always be distinguished from those of eosinophil and special myelocytes, even though they are subject to slight changes in size and shape. My observations on the mast leucocytes of the rabbit, which were carried on under the direction of Professor Downey, and to whom I wish to extend my most sincere thanks, are also a further confirmation of Maximow's results.

It is a well known fact that the early myelocyte stages of eosinophiles and special cells have a primitive or 'prodromale' granulation which is decidedly basophilic when first differentiated.

³ A preliminary report was published in the Proceedings of the American Association of Anatomists, The Anatomical Record, 1914, vol. 8, no. 2.

According to Pappenheim ('12) this primitive granulation is supposed to have nothing to do with the final eosinophilic or special granulation which is developed later. Pappenheim believes that this primitive granulation is basophilic, but that it disappears when the specific granulation develops later. The latter is also basophilic when it first forms. According to Maximow ('13) the primitive granulation is azurophilic. Downey has shown that in the guinea-pig histogenous mast cells are derived from a type of cell similar to the clasmatocyte with a primitive granulation. Whether the primitive granulation disappears or becomes the final mast cell granulation is not known.

Maximow and Pappenheim have called attention to the very decided basophilic quota of young eosinophil and special granules in the eosinophiles and special cells of the rabbit. Bone-marrow of the rabbit, prepared according to Pappenheim's⁴ method, show the preponderance of basophilic granules in eosinophil and special myelocytes very well. The granules are seen to vary in size, but are generally rounded or slightly irregular and show no definite arrangement within the cell body. All of the granules when first formed have a strong affinity for basic aniline dyes, in which respect they resemble the basophilic granules of mast cells. Other cells, however, whose general character is similar to these contain a few granules which are intermediate in staining reaction, having an affinity for both the acid and basic component of the staining mixture which gives these granules a mixed tone. Cells can also be found in which the number of basophilic granules is greatly reduced with a corresponding increase in the number of the intermediate granules. This change of staining reaction in the basophilic granule suggests that the early myelocyte with basophilic granules is being differentiated into a cell in which the granules are acidophilic, and shows that granules of this type are not true mast granules.

Benacchio has made similar observations; however, he goes further and concludes that the myelocytes with basophilic granules, similar to those described above, are the only type of

⁴ *Folia Haem, Archiv.*, Bd. 13.

basophilic myelocyte present in the marrow of the rabbit; in other words, that all of the myelocytes with basophilic granules are destined to differentiate into eosinophiles and special cells.

Kardos, in working with sections of bone-marrow fixed in 100 per cent alcohol and Helly's mixture, found neither mast cells, nor cells of any kind which contained basophilic granules. Paraffin sections and smears were studied in the present investigation, but with decidedly different results from those obtained by Kardos. In sections (material fixed in 100 per cent alcohol and stained in alcoholic thionin) basophilic myelocytes are just as numerous as they are in the bone-marrow smears prepared according to Pappenheim's method. The alcoholic material shows practically the same conditions as are seen in the bone-marrow smears. Sections stained in May-Giemsa show many cells which contain basophilic granules only, while others contain both basophilic and eosinophilic granules, and in still other cells all the granules are decidedly acidophilic. Furthermore, and in direct opposition to the findings of Benacchio and Kardos, it is possible to demonstrate in these same preparations and in smears also, a second type of basophilic myelocyte in the marrow of the adult rabbit. This is the mast myelocyte or the precursor of the mast leucocyte. Scattered throughout the section one sees numerous cells which contain a variable number of granules; the granules have a remarkable avidity for basic aniline dyes. These granules are metachromatic as well as basophilic, in fact, the metachromasia of the granules is so pronounced that they can neither be over-looked nor interpreted as the ordinary basophilic granule of the eosinophil and special myelocyte. The size and shape of the metachromatic granule, and the configuration of the nucleus are very suggestive of the mast leucocyte of the blood. On closer investigation and observation their identity is at once apparent.

In the marrow of the adult rabbit, in addition to the fully differentiated mast leucocytes with a more or less polymorphous nucleus, all intermediate stages between them and their myelocytes can be followed out. In the early myelocyte stages the nucleus is round, but later it becomes polymorphous. A dis-

tinictive feature of the mast myelocyte, as pointed out by Maximow, is its very thick nuclear membrane. The writer can also add in further support of Maximow's statement, that eosinophil and special myelocytes usually occur in groups, while mast leucocytes and mast myelocytes appear more or less scattered throughout the section.

Mast myelocytes are well preserved in bone-marrow smears fixed in lucidol-acetone and stained in either alcoholic thionin or May-Giemsa. For fixation the solution devised by St. Szécsi⁵ is used. Smears of fresh bone-marrow were made by rolling a small piece of marrow over a chemically clean cover-slip. Without allowing the smears to dry in the least they were immediately placed into a covered dish containing the lucidol-acetone fixative. At the expiration of fifteen minutes the smears were removed from the lucidol-acetone mixture, transferred without drying to another covered dish containing a mixture of acetone and xylol, three parts of the former to two parts of the latter. St. Szécsi states that the object of using this mixture is to dissolve the lucidol crystals, and clear the preparations, ten minutes are sufficient to complete the process. Finally the smears are placed in methyl alcohol, from one-half to one minute. Bone-marrow smears, provided that the smear is not too thick, are well fixed after being subjected to the action of the lucidol-acetone.

In view of the fact that several modern haematologists have denied the presence of mast leucocytes in the bone-marrow of the rabbit, the lucidol-acetone preparations are of particular value and interest. After seeing a single preparation there can be no doubt as to the presence of mast myelocytes and mast leucocytes in the marrow of this animal. A single preparation usually shows great numbers of mast cells. In a single field I have often counted as many as six fully differentiated mast leucocytes.

The mast myelocyte is such a distinctive type of cell that it is easily distinguished from eosinophil and special myelocytes. In lucidol-acetone preparations stained with May-Giemsa the granules of mast leucocytes stain an intense bluish black, while the

⁵ His method of procedure appeared in the *Deutschen Medizinischen Wochenschrift*, no. 33, 1913.

basophilic granules of eosinophiles and special cells are of a reddish black tinge. The sharp contrast in the staining reactions of the mast myelocyte as compared with the eosinophil and special myelocyte is so pronounced and so characteristic that every mast cell is easily separated from the eosinophil or special myelocyte.

Of the various methods tried none gave sharper pictures for the demonstration of mast cells than did the lucidol-acetone fixation. The basophilic granules of the mast leucocyte are very well preserved. In some instances the cell body is so filled with the basophilic, metachromatic granules that the outline of the nucleus is extremely difficult to follow.⁶ In cases, however, where the exact outline can be seen, I find that the nucleus is typically polymorphous and shows no similarity to a lymphocyte nucleus, neither does it possess lymphocyte characters nor show signs of degeneration. My preparations showed nothing to support Pröscher's theory that the mast leucocyte is derived from a lymphocyte and that the nucleus remains practically identical with the lymphocyte nucleus. In all probability Pröscher based his theory on the early, basophilic, mononuclear myelocytes of eosinophiles and special cells. At any rate, the technique which he used was such that the granules of mast leucocytes would not be preserved.

The lucidol-acetone preparations show further that the basophilic granules of mast leucocytes vary in form, size, and in number as previously stated. In the rabbit the granules are fine, usually rounded or slightly irregular. As far as the mast leucocyte of the rabbit is concerned there is no evidence to show that the nucleus is concerned in the elaboration of the granules, as is claimed by Weidenreich for the mast leucocyte of man. Pröscher also claims that the nucleus takes no active part in the elaboration of granules.

Maximow's method of fixing bone-marrow in 100 per cent alcohol followed by staining in alcoholic thionin or May-Grünwald was also tried. These preparations also show that the

⁶ A more detailed description of these cells, with figures, will appear in the final publication (*Folia Haematologica*).

mast leucocytes are present in the marrow and that the staining reactions are very characteristic. It is not the object of the writer to re-describe Maximow's results with this method.

In previous work on the mast leucocytes of the rabbit, Maximow has called particular attention to the fact that the granules of these cells are extremely soluble in water, and has cautioned against using watery fixatives and watery stains. The writer found that after fixation in Helly's mixture no mast granules could be detected with any of the various stains used. This would indicate that the mast granules are soluble in water. However, after alcohol and lucidol-acetone fixation, the granules are able to resist the short exposure to water to which they are subjected while being stained in the Giemsa solution. In the material fixed in Helly's mixture, however, the granules are exposed to the action of water for a long period of time which is sufficient to dissolve them. It is obvious that only those methods of technique which preserve the granules will be of real value in determining the origin of mast leucocytes, since the cells are difficult to recognize after their granules have been dissolved out. Little heed has been given to Maximow's repeated warning as to the solubility of the granules in water, and in all probability this accounts for the fact that Benacchio and others have failed to find mast leucocytes in the marrow of the rabbit.

SUMMARY

The bone-marrow of the rabbit contains true mast myelocytes with basophilic granules in addition to the myelocytes of eosinophiles and special leucocytes whose granules are also basophilic. With ordinary methods all of the myelocytes with basophilic granules seem to belong to the latter two types of leucocytes, but after fixation in alcohol, or better in lucidol-acetone, the granules of the true mast leucocytes are also preserved. Their distinctive characters are such that they can always be distinguished from the basophilic granules of the eosinophil and special myelocytes.

The general life history of the mast leucocyte runs parallel to that of the other granular leucocytes of the bone-marrow. Their granules are differentiated gradually out of the basophilic cytoplasm of mononuclear cells. The granules are strongly basophilic from the moment of their first appearance and remain so throughout the life-history of the cell. As the number of granules increase the nucleus gradually changes shape, becoming distinctly polymorphous in the fully differentiated cell.

Fully differentiated mononuclear mast leucocytes are never found in the blood or marrow of the adult rabbit. These cells, therefore, do not show the relationships to lymphocytes of the circulation described by Pröscher and others, and they are never differentiated from the lymphocytes of the circulating blood in the normal animal.

When the proper methods of fixation have been used the mast leucocytes of the rabbit show no evidence whatever of degenerative changes. Their granules are, therefore, not products of a mucoid degeneration of the spongioplasm of lymphocytes (Pröscher, Pappenheim and others), but are formed by the progressive differentiation of the cytoplasm of mononuclear cells of the bone-marrow.

The haematogenous mast cells of the rabbit form a distinct and independent line of granulocytes which is in no way related to the eosinophil or special leucocytes excepting through the non-granular parent-cell of the bone-marrow.

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A NOTE ON THE COURSE AND DISTRIBUTION OF THE NERVUS TERMINALIS IN MAN

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TWO FIGURES

Johnston ('13) was the first observer to determine the presence of the nervus terminalis in man. He first reported its occurrence in human embryos and later ('14) described the nerve for the adult. Brookover ('14), working independently, also observed the presence of this nerve in adult man. Apparently the material used by these authors permitted only of the examination of a portion of the intracranial course of this nerve. It is the purpose of the present paper to report observations on the intracranial course and nasal distribution of the nervus terminalis in man.

The observations about to be reported are based on gross dissections of prepared specimens of the heads of several human fetuses varying in age from ten weeks to the newborn. Two adult heads were examined. The nervus terminalis was identified in all the specimens. Drawings were made from the two most favorable dissections. Figures 1 and 2 represent such drawings. The former represents the medial sagittal dissection of the head of a six-months human fetus, the latter a similar dissection of a ten-weeks human fetus. For purposes of dissection the specimens were prepared as described by the writer ('12) in a previous communication.

The intracranial portion of the nervus terminalis, as shown in figure 1, appears on the surface of the brain in the region of the olfactory trigone and courses anteriorly over the medial surface of the olfactory tract and bulb and on to the lateral surface of the crista galli, to pass through foramina in the cribri-

form plate well forward. In its course over the medial surface of the olfactory tract it will be seen that the nerve forms a compact bundle of nerve fibers. On the medial surface of the olfactory bulb, however, it breaks up into a close plexus of fibers intimately associated with the fila olfactoria. It forms

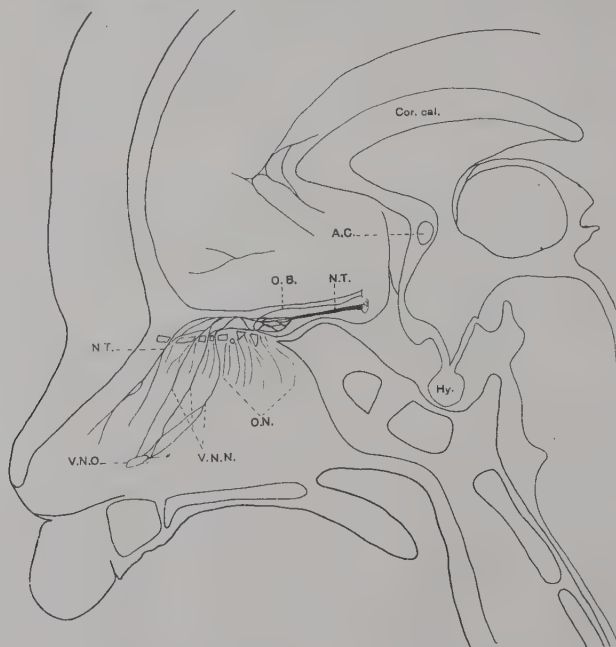


Fig. 1 Medial section of the head of a six-months human fetus with the nasal septum removed, showing the origin, course and distribution of the nervus terminalis. *Cor. Cal.*, corpus callosum; *A.C.*, anterior commissure; *N.T.*, nervus terminalis; *O.B.*, olfactory bulb; *O.N.*, olfactory nerves; *V.N.N.*, vomero-nasal nerves; *V.N.O.*, vomero-nasal organ; *Hy.*, hypophysis.

a loose plexus on the lateral surface of the crista galli imbedded in the layers of the dura mater. In this position the separated filaments of the nervus terminalis lie some distance dorsal to the cribriform plate of the ethmoid bone instead of lying directly on its upper surface as do the fila olfactoria. In the specimens examined the height to which the nerve attains on the lateral surface of the crista galli or the amount of arching upward of

the filaments of the nervus terminalis in this region, depends apparently upon the degree of development of the crista galli.

The distribution of the nervus terminalis to the nasal septal mucosa is similar to that described by Huber and Guild ('13) for the rabbit. Within the cranium filaments of the nervus terminalis join the olfactory and the vomero-nasal nerves and

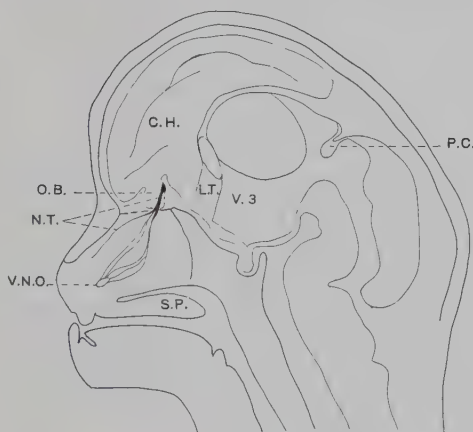


Fig. 2 Medial section of the head of a 4.5 cm. human embryo, with the nasal septum removed to show the origin, course and distribution of the nervus terminalis. *C.H.*, cerebral hemisphere; *L.T.*, lamina terminalis; *N.T.*, nervus terminalis; *P.C.*, posterior commissure; *O.B.*, olfactory bulb; *S.P.*, soft palate; *V.3*, third ventricle; *V.N.O.*, vomero-nasal organ.

apparently pass to the septal mucosa with them. The majority of the fibers, however, form a single strand and pass through the cribriform plate anterior to the exit of the vomero-nasal nerves. Upon reaching the nasal cavity the nervus terminalis takes a path anterior to that of the vomero-nasal nerves, lying just posterior to the antero-superior border of the nasal septum. In figure 1 it is represented as breaking up into three main filaments which can be traced downward nearly to the level of the vomero-nasal organ. In the first part of its nasal course it is joined by a small filament from the medial nasal branch of the anterior ethmoid nerve.

In figure 2 the long axis of the olfactory tract and bulb occupies a plane approaching the perpendicular instead of the horizontal, as is shown in figure 1. The nervus terminalis appears on the surface of the brain in relatively the same position as in figure 1 and passes directly downward to the cribriform area where it lies in close proximity to the vomero-nasal nerves. After sending a few strands to accompany the vomero-nasal nerves the larger portion of the nervus terminalis passes through the cribriform area and is distributed to the septal mucosa anterior to the path of the vomero-nasal nerves.

In conclusion it may be stated that on account of the relation of the nervus terminalis to the crista galli, where the latter is sufficiently developed to cause a stretching out, as it were, of the overlying dura mater with its contained nerve, the continuity is here usually lost in gross dissections and the fibers associated with the vomero-nasal and olfactory nerves alone remain to determine its distribution to the septal mucosa.

The distribution of the nervus terminalis in man as in the rabbit is mainly to the mucosa of the nasal septum anterior to the path of the vomero-nasal nerves. Their ultimate terminations could not be determined.

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ON WEBER'S METHOD OF RECONSTRUCTION AND ITS APPLICATION TO CURVED SURFACES

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FIVE FIGURES

In embryological work it is sometimes a matter of importance to determine with accuracy areas of epithelial thickening which are not demonstrated satisfactorily by the ordinary methods of graphic or plastic reconstruction. Placodal thickenings of the skin ectoderm, thickened zones in the neural tube, and the thickened areas found in the early archenteron are examples of structures which are not well demonstrated by these customary methods. A method of reconstruction which brings out graphically the extent and comparative thickness of such areas was devised some years ago by A. Weber, and was used by him with much success in the study of the very early development of the great glands of the digestive tract. Weber first published an account of his method in 1902,¹ and again a shorter summary in connection with the final report on his work in the following year.² Apparently the method has not been employed elsewhere. I have found it of such interest in the study of the earlier stages of the pancreas and liver that it has seemed desirable to present it here with some modifications which may be found useful, particularly in its application to curved surfaces.

Weber's method is based upon that of graphic reconstruction from transverse sections. An outline (from either lateral or dorsal view, as desired) of the organ to be reconstructed is plotted

¹ Une méthode de reconstruction graphique d'épaisseurs et quelques-unes de ses applications à l'embryologie. *Bibliog. Anat.*, T. 11.

² L'origine des glandes annexes de l'intestine moyen chez les vertébrés. *Arch. d'Anat. Micr.*, T. 10.

out on transverse section lines in the customary manner. Instead, however, of completing the reconstruction by indicating the *contour* of the surface thus outlined (as is commonly done), the *thickness* of the wall which forms that surface is measured in the transverse sections, and the variations in this thickness are plotted out upon the reconstruction. The reconstruction will then bear a number of lines which mark the boundaries between areas of epithelium of different thicknesses. To finish the reconstruction, the areas thus outlined are filled in with different shades of a single color, the darker shades being used for the thicker areas of the epithelial wall. The finished reconstruction then will exhibit the outline of the structure and the variation in the thickness of the part of its wall which is shown in this particular view. It will give no conception of the surface modeling of this wall aside from what can be determined from the outline alone.

The picture obtained is much the same as would be secured were it possible to remove the wall of the structure, render it translucent and, magnifying it greatly, hold it before a bright light. The thicker portions of the wall would then appear to the observer as darker areas, as they do in the reconstruction.

Figure 1 is a graphic reconstruction of the left side of the archenteron of an embryo of *Torpedo ocellata*, 4.0 mm. in length (No. 765 of the Harvard Embryological Collection). The portion of the gut lying on either side of the anterior intestinal portal and included between the lines *A* and *B* has been reconstructed by Weber's method and is shown in figure 4. The latter figure shows by means of its coloring a broad band of thickened epithelium extending dorso-ventrally across the gut at the level of the anterior intestinal portal. The lower and thickest part of this band represents the anlage of the gall bladder and liver, while the upper part includes the future pancreatic region. A thickened spur extends backward from this zone, and marks off the line along which the intestine will eventually separate from the yolk-stalk ventral to it.

The method of preparation of such reconstructions can best be explained in detail by following an example of the process.

For this purpose I will use the reconstruction shown in figure 4 which has just been described.

In preparing this reconstruction, drawings were made of each section of the portion of the gut involved, although fairly satisfactory results can be secured with drawings of every other section. These drawings should be made at a high magnification, preferably over 300 diameters. As the sections are drawn, they show of course the curvatures of the contour of the archenteron wall. It is desirable to eliminate these curvatures in the reconstruction and to present the gut wall as an approxi-

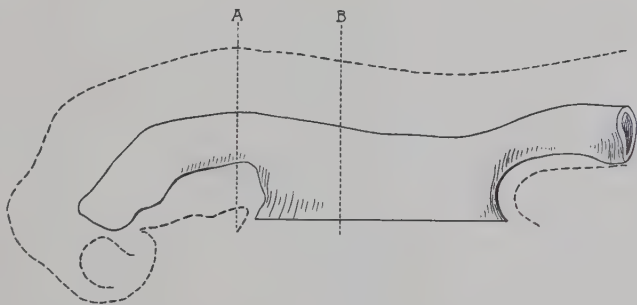


Fig. 1 A graphic reconstruction (lateral view) of a portion of the archenteron of an embryo of *Torpedo ocellata* 4.0 mm. long (H.E.C. 765). $\times 30$. The outline of the embryo is represented in broken line. The archenteron is drawn in solid line. The portion of the archenteron lying between the dotted vertical lines A and B is shown in a Weber's reconstruction, at higher magnification, in figure 4.

mately flat plane. If this is not done, areas which project sharply from the general plane of the archenteron will be represented in the reconstruction as much narrower than they actually are in the specimen or, if small, may be lost entirely. To eliminate these curvatures, it is necessary to divide the outer margin of each section into a number of short cords or segments, each of which will be comparatively straight, and to lay off segments of an equal length on the transverse section lines of the reconstruction. This can be done with a pair of small screw compasses. For work at a magnification of 300 diameters and over,

segments of 1 cm. are small enough to eliminate most of the error from curvature. Figure 2 is of a section (No. 104) of the reconstruction under discussion. The short lines on the inner side of its margin represent the boundaries of these centimeter segments.

The thickness of the epithelium forming the gut wall is now to be measured in each drawing. For this purpose a unit of measurement must first be determined. Working with drawings made at a magnification of 300, it has been found that the 1.5 mm. is the smallest unit practicable for such a scale. By using this unit one is able to measure variations of less than 5 micra in the thickness of the gut wall, and errors of projection and drawing would probably render more refined measurements of little value. A scale of 1.5 mm. units is laid out upon a stiff card, or better, a piece of transparent celluloid. This scale or gauge is then passed over each section, care being taken to keep its graduated margin at right angles to the *axis* of each segment of the drawing and its zero point at the inner margin of the epithelium. This process is begun at the dorsal median line on each section and is carried laterally or ventrally from that point. At the first place where the thickness of the epithelium is found to correspond to a graduation point on the scale, a fine line is drawn out to the side of the section and the thickness noted at its end. The scale is carried along the section until a point is reached where the thickness of the epithelium corresponds with the next graduation (either above or below the former one) on the scale. Again a line is drawn out from the section at this point and the thickness noted. This process is continued until the entire side of the section has been gauged and marked off into segments in which the variation in thickness is not over 1.5 mm. on the drawing or 5 micra in the corresponding section. An example of a section thus gauged is shown in figure 2. As shown by the gauge lines, the epithelium immediately below the dorsal median line is between 30 and 25 micra (6.5 mm. at $\times 300$) in thickness. The thickness falls to 25 micra at the point indicated. This is followed by a broad zone which is less than 25, but over 20 micra thick. Ventral to this zone, the epithelium increases to

a thickness of between 35 and 40 micra, and then again decreases to less than 10 micra as it approaches the blastoderm. The arrows seen in the figure point towards the thinner edge of the segment and are used to avoid confusion in mapping out the gauge lines on the reconstruction at a later time. It is impor-

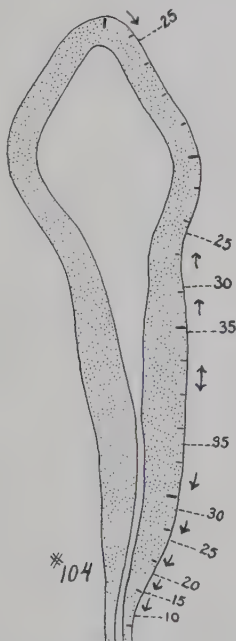


Fig. 2 Drawing of a transverse section of the archenteron of a Torpedo embryo 4.0 mm. long (H.E.C. 765) showing the method of measuring sections for reconstruction by Weber's method. The short lines extending into the section mark the centimeter segments used in eliminating lateral curvature from the section. The longer lines extending out to the right from the section are the gauge lines. The figures at the end of the gauge lines indicate in micra the thickness of the epithelium at the points touched by them.

tant that in gauging the section drawing the scale be held at right angles to the long axis of that particular part of the wall which is being measured rather than at a similar angle to either the inner or outer surface of the epithelial strip. This holds particularly when gauging the thickness of an epithelial band which

is rapidly changing in caliber, or when gauging a portion of a band which forms a sharp curve. Failure to observe this precaution causes noticeable error both in the gauge readings and in the position of the gauge points on the drawing. There will often be encountered considerable portions of the wall, the thickness of which corresponds exactly to the gauge unit or a multiple of it. My rule, made arbitrarily, has been to mark as the gauge point the first (i.e., most dorsal) level at which such a zone is encountered.

A reconstruction outline is now made on section-lined paper in the usual manner, except that only the dorsal margin of the gut is mapped out. Using this margin line as a base, the transverse section lines are divided into centimeter segments corresponding to those made on the margins of the drawings of the sections. In practice it is convenient to mark the point separating each block of 5 cm. segments in a different color to aid in plotting. The ventral margin of the gut and the gauge points, which have been determined on the cross section drawings, are now plotted on the transverse lines of the reconstruction in the usual manner, except that instead of measuring the distance of each point from the dorsal margin of the section and transferring this measurement to the corresponding section line as is customary, one measures the distance of the point from the nearest centimeter mark in each case. The ventral outline of the reconstruction and the gauge lines indicating the thickness of the epithelium are established by connecting all the points of the same order as is done in an ordinary graphic reconstruction. The reconstruction will now have the form seen in figure 3. There the base and transverse reconstruction lines are lightly drawn and the centimeter segment points are indicated as small dots on the latter. The outline of the reconstruction is indicated in heavy line. The ventral margin posterior to the anterior intestinal portal has been cut away arbitrarily in a straight line. The gauge lines indicating the thickness of the epithelial wall of the organ are represented in heavy broken line. The vertical figures placed at the termination of the gauge lines indicate their values in micra.

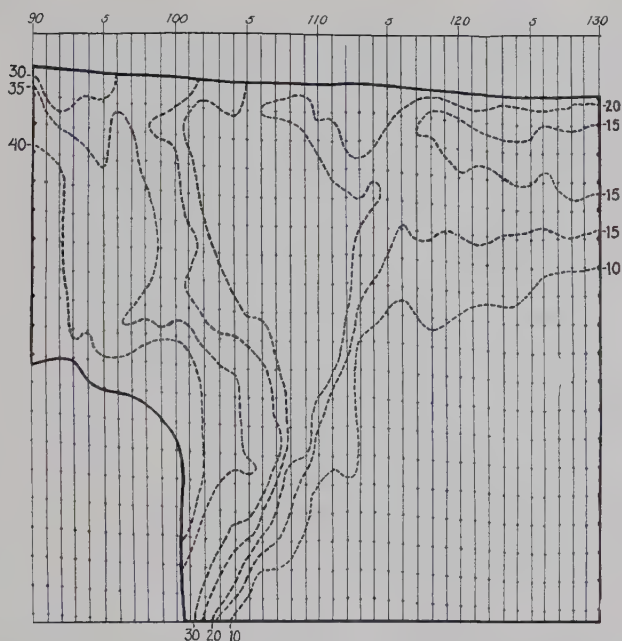


Fig. 3 Reconstruction plat of a Weber's reconstruction (lateral view) of the portion of the archenteron bounded by the lines *A* and *B* in figure 1. The light vertical lines represent the transverse sections. The outline of the reconstruct on is represented in heavy solid lines. The gauge lines bounding areas of epithelium of different thicknesses are represented in heavy broken lines. The dots on the transverse lines represent the boundaries of the centimeter segments described in the text and shown in figure 2. The vertical figures at the margins of the reconstruction give the value of the gauge lines in micra. The figure is reduced to one-half the size of the original reconstruction, which was made at a magnification of 350.

There remains but to color in the areas which are marked off by the gauge lines. Weber did this with water-color and secured excellent results, as his figures show, but a much simpler and quite as satisfactory a method is to use papers of different shades of gray for the different areas. Such papers should be as near 'pure' mixtures of black and white as it is possible to secure. The only kind which I have found satisfactory is the 'Herring' series which has been prepared for color work in psychological

laboratories. The shades numbered 3, 4, 7, 10, 16, 20, 30, 42 and 46 make a satisfactory series of marked but fairly equal gradations.

To build up the final colored reconstruction gray papers are selected, equal in number to the areas mapped off on the reconstruction by the gauge lines. The entire outline of the reconstruction is now traced upon the lightest colored paper. This area is cut out and pasted firmly upon a piece of compo or plaster board. Upon the paper of the next darker shade there is traced an outline similar to the preceding except that the space representing the thinnest area is cut away. This second sheet is pasted upon the first one so that the similar angles and sides correspond. In this way the area of thinnest epithelium is represented by the lighter colored paper and all thicker areas by the darker one. This process is continued, all thinner areas being cut away from each new outline until the darkest and final shade of paper will have the shape and will represent the area of the thickest epithelium only. This method of building up the papers of different colors in strata will be found much easier than to cut out each separately and then attempt to fit them together in a mosaic. Figure 4 is a half-tone made directly from such a colored reconstruction and based upon the plotting illustrated in figure 3.

The example just described is of a lateral view reconstruction. Reconstructions may be made by Weber's method to show dorsal or ventral views of epithelial structures as well. For this purpose the transverse sections of the structure are drawn and measured in the same manner as that described. A reconstruction plat is then laid out as follows. A vertical line is drawn in the middle of the sheet to represent the dorsal median line of the structure and transverse section lines are drawn on either side at the proper distance apart and at right angles to the median vertical one. The centimeter points, which on lateral view reconstructions must be measured off on each transverse section line, can be located on the plat in this case by simply ruling vertical lines parallel to and at centimeter intervals from the median one. The gauge points and lines marking the boundaries



Fig. 4 A finished Weber's reconstruction (lateral view) made from the plat represented in figure 3 and including that part of the archenteron lying between the lines A and B in figure 1. The thicknesses of the epithelium in several parts of the reconstruction are represented by the various shades of gray. Starting with the lightest, these several shades represent the following epithelial thicknesses; (1) below 10μ ; (2) 10 to 15μ ; (3) 15 to 20μ ; (4) 20 to 25μ ; (5) 25 to 30μ ; (6) 30 to 35μ ; (7) 35 to 40μ ; (8) above 40μ . This figure is reduced to three-fifths the size of the original reconstruction.

between areas of different epithelial thickness are mapped out as in the lateral-view reconstruction. Should the reconstruction be of a tubular structure, the ventral median line must be determined in each cross section drawing. In reconstructing, the tube is then represented as split along its ventral median line and flattened out laterally; i.e., the lateral margins of the reconstruction represent in fact the ventral median line of the original tube. Figure 5 is an example of such a reconstruction plat made from the same object employed for the lateral view reconstruction already described. The method of representation and lettering are the same as used for figure 3.

Several of the possible uses and advantages of this method of reconstruction have been mentioned. It remains to speak of a few disadvantages and sources of error. In the first place the outlines of the reconstructions, aside from the one used as a base line, are not strictly such as would be secured by the ordinary graphic or plastic methods. In eliminating the lateral curvatures from the transverse sections, the dimensions of the figure are increased dorso-ventrally or laterally, as the case may be, without a similar increase antero-posteriorly. Weber made no attempt to eliminate the curvature seen in cross sections, regarding it in fact as of some value in indicating contour. I have already pointed out the disadvantage in reconstructing without making this correction, which I think should be done even at the expense of some accuracy of outline, which can easily be determined by the other reconstruction methods.

While the error introduced by this method is small when dealing with structures having surfaces approximating those of cones or cylinders, it is considerable when applied to spherical surfaces. Spherical surfaces do not admit of being spread out into planes as do those of cones and cylinders. Cartographers, who have much this same problem in representing large areas of the globe, have developed a number of methods of projection to meet, in part, this difficulty. These are, however, too complex for our work and are all based upon representations of the perfect sphere. For the purpose at hand surfaces which approximate spherical ones can best be treated by first compensating for the vertical curvature by the method of segmenting the outline of the section already described; and, second, by allowing for longitudinal or horizontal curvature by increasing the distance separating the cross section lines on the reconstruction plat. The latter can be done by determining the actual length of the outline of the structure to be reconstructed and dividing this distance by the number of sections which the structure contains. Multiplying the figure thus secured by the magnification at which the reconstruction outlines are drawn gives one the distance which should separate the section lines on the reconstruction plat. This practice differs from that of ordinary reconstruction in

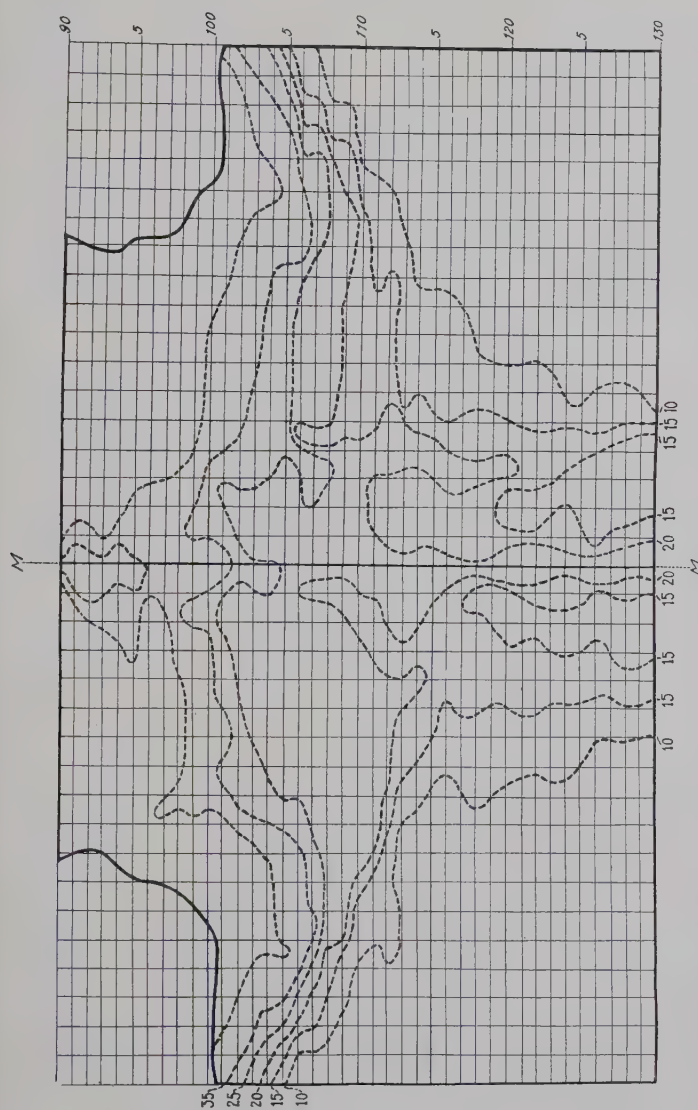


Fig. 5 Reconstruction plot of a dorsal view reconstruction of the same object as shown in figures 1, 3 and 4. The line $M-M$ represents the dorsal median line of the archenteron. The lighter lines parallel to it are the boundaries of the centimeter spaces. Scheme of drawing and lettering corresponds to that of figure 3. The scale of magnification is the same as for figure 3.

that the longitudinal axis of the structure is used in the customary method instead of the actual length of the outline as in this case. Reconstructions made with these corrections will show approximately the area and shape of any given outline upon a curved surface, although neither will be strictly accurate. As a rule such correction will be unnecessary unless one is dealing with surfaces which curve very abruptly. Measurements of the thickness of epithelial plates which curve longitudinally or horizontally will always be a little exaggerated because such plates are cut somewhat obliquely by transverse sections. There seems to be no practical way of eliminating this error.

Finally, the reconstruction will represent the variations in thickness of the epithelium as occurring in definite steps and not as gradual transitions as in nature. Most of this artificial distinction can be eliminated by using the smallest unit of measurement practicable and thus increasing the number of shades present in the reconstruction while decreasing the degree of their difference. Great reduction of figures in their reproduction also aids in securing the effect of gradual transition.

ON THE STRUCTURE OF THE ERYTHROCYTE

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FOUR FIGURES

This paper was undertaken with the hope of contributing something as to the structure of the so-called stroma of erythrocytes and as to the presence and character of a capsule or cell-membrane belonging to them.

The fact that the mammalian red blood corpuscle upon losing its nucleus becomes biconcave, its peripheral ring remaining thicker than its central region from which the nucleus has been lost, has always suggested the existence of a supporting framework. Were the corpuscle merely a hemoglobin-carrying sac borne in the blood stream, the natural tendency would be for it to assume a spherical form, the pressure on all sides being equal. The mammalian erythrocyte after being carried through capillaries smaller than its diameter, and after being drawn around angular curves of capillaries, which may stretch and modify its form considerably, always resumes its original form when returned to larger capillaries. This not only suggests a supporting frame-work for its content but also that this framework is plastic or even possesses certain elasticity.

The question of a membrane about the corpuscle is most disputed in the discussions. The most frequently advanced theory against the existence of such is that the corpuscle possesses no membrane at all but is merely covered by a film of lecithin or other lipid substance acquired from its environment, and Norris (Physiology and pathology of blood '82) even suggests evidence that fluid droplets enclosed by lipoids (myelin) tend to assume flattened shapes, while when enclosed by films of ordinary fats they are invariably spherical. The very probable presence of a film of some lipid or fat surrounding the entire corpuscle is

seldom disputed and is accepted here. This is indicated by and explains the phenomena of forming into rouleaux and is supported by other arguments as well. But the presence of such a film need not disprove the existence of a membrane or capsule as a structure belonging to the corpuscle itself and which may be covered without by a film of lipoid substance. Fluid droplets enclosed in lipoid or fat films when shaken will become divided into smaller droplets and droplets of greatly varying size, whereas the erythrocytes of normal blood are strikingly uniform in size and while shaking them may break up or burst many of them, fragments resulting do not assume the form of the original corpuscle. Further, it seems very improbable that a film of lipoids alone would result in the folded and crumpled appearances shown by the so-called 'crenated corpuscles' resulting from extraction of a portion of the content. On the contrary, it would seem that such a film, still in the blood plasma, would merely thicken instead of becoming folded. Also, crenated corpuscles have lost the biconcave form, do not have the flattened shape suggested by Norris for fluid droplets surrounded by a film of lecithin. Crenation is suggested here as indicating the presence of a membrane intrinsic to the corpuscle which has become folded due to partial extraction of the original content by exosmosis, the crenated corpuscle becoming approximately spherical, due to a disturbance or destruction of its original internal structure by the process. The chemical compound, hemoglobin, so far as is known, has no anatomical structure. It is a complex organic compound in solution capable of various degrees of dilution. The loss of the biconcave form in crenation and the assumption of the spherical form in swelling from the action of distilled water, for example, suggest the destruction of a framework in which the hemoglobin was supported and by which the biconcave form of the nonnucleated corpuscle was maintained.

The original cell, the typical cell, possesses a framework of spongioplasmic filaments, a cytoplasmic and karyoplasmic reticulum, in the meshes of which are the more fluid portions (hyaloplasm) and the various forms of granules comprising the structure and content of the cell. In the functional differentiation

of certain cells a marked increase in the evidence of a cytoplasmic reticulum is well known. The neuro-fibrillae of the nerve cell, for example, consist of anastomosing filaments more abundant and more evident than in the germinal or neuroblast stage of this element. Certain functioning gland cells show a reticulum in their cytoplasm. Hardesty ('05) and Nemiloff ('10) have shown that the emulsion comprising the medullary sheath of the nerve fiber has its component globules suspended and supported by a delicate reticulum, Hardesty showing this reticulum to be continuous into the membrane or neurilemma without and into a thinner bounding membrane (axolemma) about the axone of the nerve fiber, and that this framework and these two membranes, and not the fat of the sheath, maintain the shape of the sheath. He considered the membranes of the sheath as condensations of the internal framework. It is very probable that the corpuscle of adipose tissue, the fat cell, possesses an internal framework continuous with its capsule, both maintaining its general shape. Schäfer (vol. 2, part 1, Quain's Anatomy, 11th edition, '12) cites the fact that an erythrocyte, that of the newt for example, may be cut into two without resulting exudation of its content. Like the medullary sheath, the muscle fiber, the nerve cell or the fat corpuscle, the erythrocyte is an example of an especially differentiated arrangement for the performance of a special function.

The findings in the literature dealing with the structure of the red blood corpuscles seem to vary according to the methods of preparation used by the various authors and according to the different interpretations of the results obtained by them. Of the very voluminous literature, many of the papers consulted indicate a lack of familiarity with histology in general and an incompleteness of investigation to an extent that they seem of no value here. However, some authors give definite statements as to what they deem the structure of the erythrocyte and a number have considered the subject thoroughly.

Renant in his Histology ('89-'93) says red blood corpuscles possess no true cell-membrane, but merely a peripheral condensation of the cytoplasm such as that formed about a cell when

exposed to air. Von Ebner (in Kölliker's Handbook, '02) states that a membrane must be assumed on the surface of the erythrocyte, a membrane insoluble in water and which allows osmosis, but which cannot be called a membrane in the ordinary sense and may be similar to the exoplasm of other cells. Löhner ('07) believes that a true histological membrane, similar to a cell membrane, is improbable. His method of preparation of the corpuscles for study consisted in crushing mammalian corpuscles on the slide. He also tried drying them in indifferent substances. He describes them as jelly-like and elastic in character with a thinner, more compact peripheral layer and a broader less compact inner region, similar to the exoplasm and endoplasm of Protozoa. If the outer compact layer is referred to as exoplasm, he thinks the superficial part of this may be termed a physical membrane or plasma-film. Dehler ('95) studied the red corpuscles of the chick, fixing them in sublimate solution and staining with iron-hematoxylin. He described the convex cells as possessing a sharp border or rind of .25 to .5 μ in thickness. Heidenhain ('96), working with the red blood corpuscles of *Proteus*, using the same method as Dehler, found the same appearances. Nicholas ('96), likewise using the same procedure, found the sharp border manifest on the erythrocytes of the chick, salamander, triton and viper.

Meves ('04 and '06) studied amphibian red blood corpuscles (frog and salamander). After condemning the technique used by Weidenreich and showing the results obtained by the latter were artifacts, he describes a very complicated procedure used by himself. In general this consisted of dried smears on the slide subjected to warmth for 30 minutes, then subjected to Flemming's fluid (weak formula) plus 1 per cent sodium chloride, washed in running water, stained with safranin, hematoxylin and safranin and Gentian orange, decolorized with neutral alcohol, and cleared and differentiated in clove oil. He describes the corpuscles as showing circumferential fibrils, which were disposed either in an arrangement parallel to the surface or in the form of a continuous skein, and radial fibrils running, some from the periphery toward the center of the corpuscle and some crossing each other, form-

ing a net. In some cases the net-work arose only in part from the peripheral arrangement. He refers to the circumferential fibrils as a membrane which is continuous with the fibrils of the network within, and he interprets the fibrils as resulting from linear arrangements of mitochondria, granules having fused to form them. He thinks the arrangement of the fibrils is similar to that described by Heidenhain for Krause's membrane in muscle fibers, the fibrils continuing into the membrane (*sarco-lemma*) in a regular system. Schäfer ('05) suggests that Meves' circumferential or peripheral fibrils merely represent a part of the reticulum of the corpuscle.

Růžička ('03 and '06) worked with frog, guinea-pig and human blood. He stained with a dilute solution of methylen blue both without and after the action of 1 per cent pyrogallie acid, which latter he thought dissolved hemoglobin. The drop of blood was mounted in normal salt solution and the methylen blue solution (0.5 gram to 1000 cc. of water) added at the edge of the cover-glass. When used, the pyrogallie acid was applied followed by the methylen blue solution. Blood from the three sources gave the same results. All showed a fine meshed reticulum with occasional knobs at the junction of its filaments. He did not think an actual membrane is present but that the corpuscle is bounded by the reticulum. The knobs at the junction of the fibrils of the reticulum being found smaller and fewer after the action of pyrogallie acid, he assumed them to represent hemoglobin. He denied the possibility that the reticulum represented a coagulation product, thinking the meshes too fine and uniform and not arranged as coagulum filaments.

In the mammalian erythrocytes, Růžička also observed the previously described large granules dispersed in the center of the corpuscle, the so-called "nucleus of the mammalian corpuscle." Quoting Löwit ('87) as claiming a character for these granules in the rabbit similar to nuclear chromatin, he claims they are only present in case of incompletely dissolved hemoglobin and are thus analagous to the larger of the knobs described at the junctions of the filaments of the reticulum and therefore are not of chromatin nature.

Bryce ('04) studied the erythrocytes of the larvae of *Lepidosiren paradoxa*. He fixed the tissues with the blood vessels containing the erythrocytes *in situ* and used sections of 10 μ . He used sublimate-acetic as a fixing fluid. This was tried in this laboratory but was found unsuited for the study of adult erythrocytes in that it precipitates hemoglobin. Bryce's illustrations show that at least in the erythrocytes of these larvae, not having acquired sufficient hemoglobin to color them, there is a reticular structure in the cytoplasm connecting with a membrane at the periphery, and he describes a meshwork or reticulum in them which was radially arranged from the nucleus to the periphery, the meshes of which in section were 3 to 4 μ in size. At the nodal points or junctions of the filaments forming the meshes he found "strongly refractile granules of considerable size" and he states that in some of the corpuscles the filaments near the nucleus appear as arranged in parallel threads, extending from the nucleus a short distance toward the periphery. He does not pass judgment on the nature of the membrane observed, but states that he was dealing with very young corpuscles.

MATERIAL AND METHODS

As is known, the *Amphiumae* carry the largest red blood corpuscles of any vertebrate as yet examined. One of the species of this animal, the *Amphiuma* means (the 'blind eel') being easily obtainable in the ditches of New Orleans, a study of the structure of its corpuscle was suggested by Professor Hardesty. Measurements of its corpuscles in the fresh gave, measured on the flat, an average of 72.9 μ in length by 44.5 μ in width. With the same technique as finally employed for those of the *Amphiuma*, erythrocytes from the alligator, frog, snake, guinea-pig and human were also prepared and studied in comparison.

Obviously, a study of the internal structure of the erythrocytes of *Amphiuma* and those of other animals, or the study of membranes probably existing, could not be accomplished in any detail except with very thin stained sections. If a structural framework existed and if a membrane possessed visible structure, to observe such, it was equally obvious that the hemoglobin car-

ried must be wholly, or at least partially removed from the specimens. Therefore any accomplishment of the purpose in mind depended largely upon the technique employed.

The greatest difficulty was encountered in finding a fixing fluid suitable for the purpose. A fluid was desired in which hemoglobin is dissolved rather than precipitated. Hemoglobin not removed or precipitated within the corpuscle of course obscures whatever other cytoplasmic structure it may possess. Further, a fixing fluid was necessary whose osmotic action results in neither appreciable shrinkage nor swelling of the corpuscles, and one the diffusion currents resulting from whose action does not break up the structural content.

Osmic acid, bichloride of mercury, chromic acid and its potassium salts precipitate hemoglobin, and fluids in which any of these act of themselves were found impossible for the results desired. It is doubtful whether, according to Růžicka ('03), pyrogallie acid is a solvent of hemoglobin. It was deemed necessary here to fix, embed and section the corpuscles, which he did not do, and no suitable fixing fluid containing pyrogallie acid has been devised. Hemoglobin is dissolved in alcohol, distilled water, acetic acid and formic acid, and formalin does not precipitate it. The action of alcohol alone distorts the corpuscle and produces shrinkage and rupture, and acetic acid, formic acid and formalin not only produce swelling but in themselves are very poor fixing agents for the structure of cells. Van Gehuchten's (Carnoy's) fluid, containing absolute alcohol, acetic acid and chloroform, was tried and found to rend the fresh corpuscles into small fragments.

Bryce obtained his results after the action of sublimate-acetic, but he was dealing with corpuscles of larvae which probably contained considerably less hemoglobin than the corpuscles of the adults whose study was here desired. After trying a number of fluids containing corrosive sublimate and acetic acid, it was decided that the action of the sublimate in all precipitated the hemoglobin. Suggestive but incomplete results were obtained with corpuscles fixed in a mixture containing 5 cc. saturated aqueous solution of bichloride of mercury, 2 cc. glacial acetic acid, 5 cc. 40 per cent formaldehyde, and 88 cc. 95 per cent alco-

hol. Preparations of nucleated corpuscles after this fluid showed a cytoplasm more or less transparent in places and a distinct membrane bounding the periphery. In the clearer places in the cytoplasm a fairly well marked reticulum could be discerned. In preparations of mammalian (non-nucleated) corpuscles such were much less indicated.

Of all the fixing fluids tried, the most satisfactory results were obtained after using a well ripened mixture containing the following parts:

Aqueous 3 per cent potassium bichromate.....	100 cc.
Commercial (40 per cent) formaldehyde.....	4 cc.
Glacial acetic acid.....	5 cc.

When first made, this fluid has the color of the bichromate solution, but if allowed to stand or if warmed it becomes a dark greenish brown, due chiefly to the oxidation of the bichromate in the resultant reactions. Our best results were, or maybe happened to be, obtained with a mixture which had been standing several weeks. The formation of formic acid is one of the results of the ripening process.

In using this fluid (and all those tried) a fairly large shell-vial was filled about half full of the fluid and, gently shaking it, the blood was dripped, drop by drop, directly from the animal into the fluid. Even by agitating the mixture while adding the blood, some coagulated clumps cannot be avoided. The corpuscles in these clumps were found not so good for study as those floating free in the fixing fluid. All finally settle upon the bottom of the vial and the fluid may be removed with a pipette or decanted. The fluid was allowed to act for twelve hours. Changing it once or twice was thought to result in better extraction of the hemoglobin.

This fluid does not require a preliminary washing of the material in water. Small paper boxes were made from ordinary thin letter paper, labels written on the sides in pencil, and the accumulated corpuscles transferred to the boxes by pipette. The boxes nearly full of fixing fluid and corpuscles were then placed in a stender dish containing 30 per cent alcohol to a depth of about

half the depth of the boxes. In 5 to 10 minutes the corpuscles settle to the bottom of the box and some of the fixing fluid may be pipetted away. Gradual dehydration is accomplished by the transfusion of the alcohol through the paper walls of the boxes. If several boxes are carried in a small stender dish, the 30 per cent alcohol should be changed during the hour. Then the 30 per cent alcohol in the stender dish was replaced with 40 per cent alcohol and so on with grades of alcohol progressively increasing by 10 per cent in strength up to 90 per cent, which was replaced with 95 per cent alcohol and this in turn by absolute. To insure complete transfusion of the different grades and the action of each upon the corpuscles, the paper boxes should remain in each alcohol at least one hour with the stender dish covered.

From the absolute alcohol, the boxes were transferred to equal parts absolute alcohol and xylol for about 30 minutes and then placed in pure xylol to complete clearing. The boxes were next placed in a dish of melted paraffin in the thermostat for two hours. Owing to the xylol present, it was found necessary to change the paraffin or transfer the boxes to another dish of paraffin during the first hour. When first in melted paraffin, the corpuscles float about and show a tendency to adhere to the sides of the box, and to avoid much of this in the final the boxes should be gently shaken a few times during the first half-hour.

The specimens were embedded, paper box and all. The corpuscles were settled in a layer at the bottom of the box, so, when the paper was pulled off, a paraffin block was obtained with them accumulated in one side.

For the sections, the ordinary Minot rotary microtome was used, set at $1\ \mu$. The large corpuscles especially were found to have settled for the most part on the flat, or with their widths parallel to the bottom of the boxes. To obtain sections cut in this plane, the paraffin block had to be arranged with its bottom surface parallel with the edge of the knife. The paraffin ribbon was of course much packed and crumpled and, of course, few if any of the sections could have been of $1\ \mu$ in thickness, but setting the microtome at $1\ \mu$ was thought to give thinnest sections

possible. The sections were straightened and fixed on the slide by the usual water-method, without using albumen fixative. After drying, the paraffin was removed with xylol, the slides transferred to absolute alcohol and then passed through the gradually descending grades of alcohol, 3 to 10 minutes in each, down to water.

Of the staining methods tried, including gentian violet with safranin, the best results were given by alizarin and toluidin blue. To distilled water was added enough of a saturated solution of the sulphalizarinate of soda in 70 per cent alcohol to make the water a straw yellow, and in this the sections were immersed for about 12 hours. The sections were then rinsed in distilled water and some slides were placed in a 0.5 per cent aqueous solution of toluidin blue to stain nuclear structures. Other slides, in order to study the region occupied by the nucleus in greater detail, were not stained with the toluidin blue at all. The stained sections were then rinsed with distilled water and dehydrated by passing through the gradually increasing strengths of alcohol, 3 to 5 minutes in each, up to absolute, cleared with xylol and mounted.

The action of the fixing fluid and the technique of embedding, etc., when carefully applied, seemed to have produced little change in the shape and size of the corpuscles of the *Amphiuma*, frog, alligator and snake. Measurements of the sections of those of the *Amphiuma*, judged truly sagittal by the shape and the position and size of the nuclei, gave an average of $69\ \mu$ long by $38.6\ \mu$ wide as compared with the average $72.9\ \mu$ long and $44.5\ \mu$ wide given by the fresh corpuscles. For the blood of all four of the animals mentioned, sections showing the evenly oval contours characteristic of the fresh corpuscles were frequent on the slides and no disturbances of interior arrangement seemed evident in these. Fragments of corpuscles and fragments of sections of them were abundant, but most all such, from their form, were evidently produced by crushing and breaking by the knife in cutting and by the crumpling of the paraffin ribbon. Fragments of sections were often more favorable for the study desired than sections of whole corpuscles. In the sections containing the

mammalian corpuscles (guinea-pig and human), there showed much more evidence of distortion both as to contour and internal arrangement.

OBSERVATIONS

The nuclei of the nucleated corpuscles could be best observed as to their position, size, form, and arrangement of chromatin in corpuscles stained whole, after fixation, without embedding. The nuclei in the corpuscles of the *Amphiuma* and alligator especially appear to consist of a tangled coil of one or more coarse rods of chromatin supported in non-chromatin substances. The coiled and tangled chromatin rods in *Amphiuma* are very much larger than those in the alligator and, in *Amphiuma* especially, the peripheral loops of the rods produce a scalloped and lobulated contour of the nuclei quite evident in whole specimens. In the thin sections of stained nuclei, the chromatin rod or rods appear cut into short segments, as shown in figures 1 and 3. A delicate membrane about the nucleus was evident in all the nucleated corpuscles examined, but could be seen only in the thin sections and best in those in which the nucleus was not stained (figs. 2 and 3, B).

A membrane about the entire red corpuscle, after the technique here employed, was distinctly present in the blood of all the animals used. In proportion to the size of the corpuscle, it appeared relatively thicker and more condensed as possessed by the mammalian corpuscles (fig. 4, human). In the thin sections of corpuscles of *Amphiuma*, this membrane, actually thicker than that of smaller corpuscles, could be resolved under oil immersion objective into an apparently parallel arrangement of very delicate threads. With the fragments of corpuscles, broken and torn by the knife in sectioning and frequently found in the preparations, the nature of the membrane could be better observed than with intact sections. Torn and broken membranes often appeared slightly frayed in the tearing and close study led to the conviction that in *Amphiuma*, at least, the membrane consists not of a condensation of concentrically arranged parallel threads, and certainly not of concentric lamellae, but instead, of a very deli-



Fig. 1 Drawings from very thin paraffin sections of erythrocytes of *Amphiuma means*, fixed in the bichromate-formalin-acetic acid mixture and stained with sodium sulphalizarinate and toluidin blue, showing the capsule, reticulum, perinuclear membrane and the coiled rods of nuclear chromatin in stained section. *A*, corpuscle sectioned on the flat; *B*, corpuscle sectioned in profile.

Fig. 2 Erythrocyte of *Amphiuma* sectioned on the flat and slightly tangentially. Same technique as in figure 1 except that the toluidin blue was omitted. Given to show the full shape of the fixed corpuscle and, especially, the perinuclear membrane and reticulum within the nucleus.

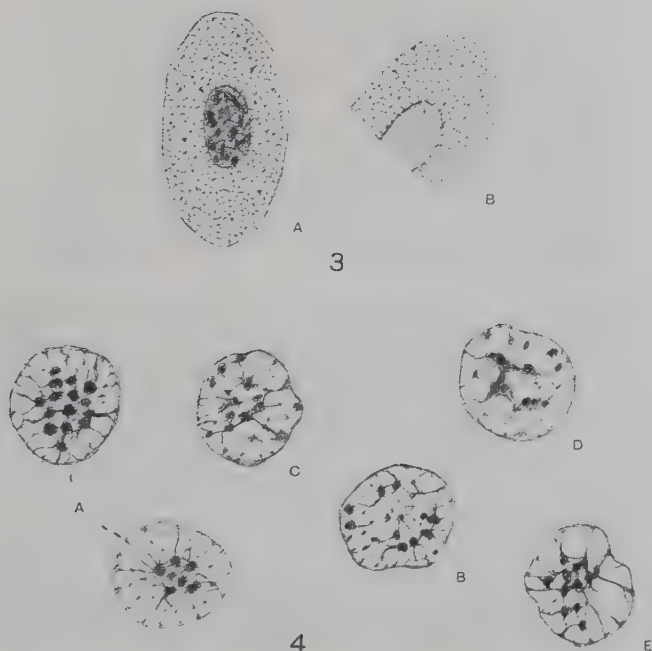


Fig. 3 From sections of erythrocytes of the alligator (*Alligator mississippiensis*). Prepared with same technique as figure 1 and drawn in same scale as figures 1 and 2 and to represent the same structures. A, section with nucleus stained by toluidin blue; B, piece of section from preparation in which toluidin blue was omitted.

Fig. 4 From sections of human erythrocytes prepared with same technique as figure 1, except that toluidin blue was not applied to the sections used for the drawing. Drawn in somewhat larger scale than figures 1 to 3. A, two sections of erythrocytes considered as representing more nearly the normal condition of the reticulum, capsule and central knots, with hemoglobin for the most part removed. B, erythrocytes with knots more distributed. C, D and E, varying degrees of rupture of the reticulum, presumably produced by diffusion currents set up by the reagents.

cate reticulum so condensed or compressed that its meshes are much elongated and thus produce the impression of a parallel arrangement (fig. 1, A). Meves, describing red blood corpuscles of frog and salamander as showing circumferential fibrils arranged either parallel to the surface or in a continuous skein, must have obtained the same appearances.

A very distinct reticulum is evident in the cytoplasmic areas of the sections and the threads of its meshes are continuous into the membrane. The meshes of this internal (cytoplasmic) reticulum, or stroma of the corpuscle, appear larger in the corpuscles of *Amphiuma* than in those of the alligator (fig. 3), frog and snake. Corpuscles of the frog and snake, not figured here, gave appearances practically identical with those given by the alligator. In the more transparent sections, those supposedly more free from hemoglobin, the fibrils of the reticulum, though very delicate and varying somewhat in size, appeared distinctly thread-like, and the meshes made by them were angular in form. That the threads of the peripheral meshes grade directly into the peripheral membrane, which itself appears as a condensed reticulum, supports the conclusion that the membrane is nothing more than a peripheral condensation of the internal reticulum and that the membrane could better be called a 'capsule' of the corpuscle. In one of his papers, Meves referred to it as a 'feltwork membrane.' This, and likewise the statement of Růžička that the corpuscle possesses no actual membrane but is bounded by the limits of a fine-meshed reticulum, seem warranted.

In preparations of corpuscles, of *Amphiuma* especially, in which the hemoglobin was obviously not so completely removed, the component threads of the internal reticulum (stroma) appeared coarser and gave the impression of being rod-like in form, and the meshes of the reticulum appeared oval instead of angular. The oval form of the mesh was due in part to larger knobs or accumulations of substance at the points of junction ('nodal points') of the threads in forming the meshes. Růžička described these knobs in his preparations of frog and mammalian blood as round in form and concluded they represented undissolved hemoglobin. Their larger size in certain preparations here and the

apparently larger size of the threads of the reticulum accompanying them is interpreted as due to unremoved hemoglobin adhering to or precipitated upon the reticulum throughout, for such preparations were always less transparent and coarser in appearance and the membrane or capsule of the corpuscle appeared dark and homogeneous as compared with those from which the hemoglobin was considered more completely removed.

All the figures here given are attempts to represent corpuscles considered as having been rendered most free from hemoglobin. The knobs, or junctions of the filaments making the reticulum, appear quite small in these, usually about as large as would be possible were two or more plastic filaments to cross each other in contact and fuse giving an increased amount of substance at their junctions, a knob, or knot of the mesh of the net. In the most clear of the preparations, occasional larger knobs occurred at the junctions of the threads. Several such are shown in figure 2. These larger knobs were usually angular or stellate with their points extending upon the threads joining in them and are interpreted as representing small masses of unremoved hemoglobin adhering upon junction points of a greater than usual number of threads.

In the corpuscles of the *Amphiuma*, alligator and frog, the threads which join or grade into the capsule at the periphery of the reticulum appear for the most part to join with the capsule at right angles and thus present here a somewhat radial arrangement. The meshes formed by these threads in joining the capsule average somewhat larger in size than elsewhere in the cytoplasm of the nucleated corpuscles. The greater amount of light admitted by these larger meshes compared with the greater density of the capsule gave the impression of a narrow, clear zone about the corpuscle just under the capsule. This was especially true with the corpuscles of *Amphiuma* (figs. 1 and 2) in which the meshes are larger throughout than in the other nucleated corpuscles examined. Meves observed this radial arrangement of the peripheral filaments of the reticulum in the corpuscles of the frog and salamander, and thought them in regular system similar to the relation of Krause's membranes in the muscle fiber.

Bryce likewise observed it in the corpuseles of lepidosiren larvae, but he considered it a part of a general radial or parallel arrangement of the filaments extending throughout from the nucleus to the periphery of the corpusele. Our preparations do not show these peripheral filaments to join the capsule in a definitely regular system but at various angles and to form meshes of varying size and shape; nor do the threads of the reticulum extend from the nucleus to the capsule in definitely radial, and certainly not in parallel, arrangements. Only in the thinnest region of the cytoplasm, in the middle of the corpusele on the flat where the nucleus is nearest the capsule, can threads be traced directly from the nucleus to the capsule (fig. 1, B, section in profile). Here the peripheral zone comprises practically the entire cytoplasm. As Schäfer ('05) suggests, these peripheral threads are only a part of the general reticulum of the corpusele. Continuous into and stretched from the capsule or membrane, they happen to appear more sparse and regular than the threads of the remainder of the reticulum.

For the larva of *Lepidosiren paradoxa*, Bryce described in each end of profile views of the corpuseles a small area free of reticulum but occupied by a number of fine dots, and he interpreted these dots as transverse sections of circumferential filaments running in the plane of the flat dimension. Our preparations showed no differences in this respect between sections cut on the flat and profile sections (fig. 1).

As noted above, the chromatin in the nuclei of the corpuseles of the *Amphiuma* and alligator appears collected into definite coiled rods instead of being scattered in granules of varying size throughout the nucleus. Preparations in which the nuclei are stained differentially do not show a sharp, dark-staining membrane bounding the confines of the nucleus as is found in certain other tissue cells where such is probably due to chromatin material being involved in or adhering upon the nuclear membrane. On the contrary, the nuclear membrane appears here to be another but thinner condensation of the reticulum or general framework, being comparable in origin and structure with the capsule, or membrane, about the corpusele. The threads of the cyto-

plasmic reticulum grade directly into it and it stains in the same way as the threads of the reticulum. It could best be studied in preparations to which a nuclear stain had not been applied (fig. 2 and fig. 3, B). Figure 2 represents a slightly tangential section of a corpuscle of *Amphiuma* on the flat. In such corpuscles, with nuclei not differentially stained, it could be noted that the threads of the reticulum grade directly into the nuclear membrane, that the meshes become suddenly smaller or more dense to produce it and that the membrane carries the same stain-reaction as the reticulum. In other words, it is here suggested that the nuclear membrane is but a peri-nuclear condensation of the general framework of the corpuscle. Sections of corpuscles in profile (fig. 1, B) show the threads to serve as continuations between the capsule and the nuclear membrane.

Furthermore, our preparations suggest that the general reticulum, or framework, is continuous into and throughout the nucleus, contributing to the support of its structures. Figures 1, A, and 2 and 3, B, are attempts to show this suggestion. In such corpuscles, the very thin sections showed the delicate filaments extending from the nuclear membrane and forming a reticulum throughout the nucleus. The meshes of this intranuclear framework seemed somewhat larger than the average of those in the cytoplasm, and especially large when containing a segment of the coiled chromatin rod. The threads stained just as those of the cytoplasm and the membranes. Knobs at the junction points of the threads could not be observed as so definite nor so large as in the cytoplasm, probably due to some extent to the necessarily more obscured nuclear area. The suggestion that the cytoplasmic reticulum is continuous and identical with the nuclear reticulum is somewhat supported by the frequently presented view that the spongioplasmic reticulum of the general cell structure is identical to (stains the same) and is continuous with the karyoplasmic reticulum, or nuclear linin.

The chemical composition of either or both of the reticula, including the membranes, may be that of a nucleo-proteid or lecithin or cholestrin, but in our preparations it occurs in the form of a network in whose meshes is supported the remaining cell load,

including the hemoglobin and the nuclear structures. Schäfer states that substances dissolving lecithin or cholestrin will produce an increase in the permeability of the membrane. Bryce thinks that the peripheral capsule ("peripheral ring or band," he calls it) is due to a condensation or massing of the meshes of the reticulum, and he suggests that the filaments of the reticulum are not necessarily fixed fibers but that they may be of colloidal nature. Taking all into consideration, he thinks the reticulum is not an artefact but an actual protoplasmic framework. Citing Bütschli's "Foam theory" of the structure of protoplasm, and noting that the meshes of the reticulum are larger than the limits given for the protoplasmic alveoli of this theory, and much larger than the meshes described for the cytoplasm of leucocytes, Bryce thinks that if the protoplasm of the erythrocyte may at one time have been alveolar in structure, the reticulum could be later derived from a vacuolated condition in which the hyaloplasm of the cell is greatly reduced and the alveolar arrangement lost by the breaking through of the walls of the alveoli. Růžicka thinks the observed reticulum is not artefact but an actual structure, that its meshes and the knobs at the junctions of the threads are not coagulation products, for the meshes are too fine and uniform and the threads are not arranged as are coagulum filaments; that the hemoglobin is carried dispersed in the meshes of the net to the periphery of the corpuscle, that the net is the vegetative part of the corpuscle and the hemoglobin the functional part.

Whatever the chemical character, our preparations seem to support the conclusion that the structures observed are not artefact, that the corpuscle is pervaded by a true reticulum, a network of threads joining each other throughout and extending in all the planes of space. That the threads of the reticulum observed serve as a supporting framework of the corpuscle and possess a certain amount of elasticity is suggested by several observations on the living red blood corpuscle: (1) the mammalian corpuscle, after losing its nucleus, remains thinner in its center from which the nucleus was lost; (2) a living corpuscle may be cut in halves and neither half suffer exudation of its content; (3) corpuscles in the circulation may be elongated and their usual

shape considerably distorted in passing through the smallest capillaries but always resume their shape upon reaching the larger vessels; and (4), in this laboratory, by tapping under the microscope the cover-glass upon fresh mounts of corpuscles of the *Amphiuma*, the nuclei could be made to shift considerably from their normal position, moving back and forth with the tapping, sometimes being slightly spread by the pressure, but, the pressure removed, they would resume their normal position and size in the center of the corpuscle floating in the plasma.

With human corpuscles, the technique here employed was not altogether as successful as with the nucleated forms. The stained sections showed more distorted contours and internally ruptured corpuscles than those of *Amphiuma*, alligator and frog blood prepared in the same way. In the latter preparations, evidences of rupture produced by the reagents used were somewhat rare. Figure 4 is given to represent appearances found in the preparations of human corpuscles. Blood from the guinea-pig gave the same appearances. The two corpuscles farthest in the left (fig. 4, A) represent the form most common in the preparations and, showing less distortion in contour, were the form considered as most nearly representing the normal. The remaining four corpuscles were selected as an attempt to illustrate, progressively, appearances of injurious effects produced by the reagents.

It may be noted that the capsule, the sharp peripheral border of the corpuscle frequently mentioned in the literature, is here relatively thicker and more densely staining than that of the *Amphiuma*. This may be due in part to a less complete extraction of the hemoglobin considered evident in the interior. The threads of the reticulum, while grading into the capsule and continuous with each other throughout the corpuscle, do not present so nearly uniform size as in the other forms studied. Filaments much thicker than others appear to radiate from the central region, while attached to these are numerous smaller threads joining throughout and completing a general reticulum. At the junction of the larger filaments with the capsule there usually appears a visible knob, or hillock of attachment. One gets the impression in close study that the smaller, probably the

normal, meshes are themselves crossed by still finer threads, while a larger mesh may appear perfectly clear. Fine knobs show at the junction or nodal points of the threads, varying in size with the size of the threads.

In the corpuscles considered more nearly normal (fig. 4, A), the frequently described large knobs dispersed in the central region of the corpuscle comprised the most prominent feature in our sections. These appeared approximately spherical in shape and uniform in size and the number observed varied from 17 to 6, a variation due no doubt in part to the planes in which the corpuscles were sectioned. In sections in which the knobs were less darkly stained, one could get the impression, under highest magnification, that these knobs themselves are fibrillar, that they are knots or condensations of very fine threads. If they carry any nuclear material (they have been called the nucleus of the mammalian erythrocyte), our sections suggested it very doubtfully that any stain reaction of a chromatin character is retained in them. Aniline nuclear dyes, such as gentian violet, and even hematoxylin may be retained in them longer than in the threads of the reticulum, but the color will wash out, and the fact that it is retained in them more deeply at times is considered due (1) to their greater compactness of mass holding more of the dye than the smaller and looser structure, but indifferently nevertheless, and (2) to unremoved hemoglobin being entangled within them. They were always darker than the filaments, but this is to be expected, due to their greater density obscuring the light. Occasionally the knobs or knots appeared more scattered throughout the corpuscle and then to vary more in size, as shown in B of figure 4.

The larger filaments, those usually described and which appear more or less radially arranged, are here considered as artefact. Corpuscles manifestly ruptured internally by the action of the reagents (fig. 4, E) show large, clear, vacuole-like meshes and these are always bounded by the thick filaments. It is suggested that these large clear meshes represent areas of the reticulum ruptured by diffusion currents of the fixing agent or alcohols in preparation, and that the broken threads of these

areas have been washed together or condensed to form the thicker filaments. Often the latter give the impression that they themselves are composed of finer threads. They occur more or less radially between the centrally placed knots and the capsule probably because these knots are the firmest fixation points.

As to the knobs or knots ('nuclei') of the central region, we beg to suggest the possibility that they may represent remains of an originally existing membrane about the nucleus and of a reticulum within the nucleus of the erythroblast, or nucleated stage of the mammalian corpuscle, similar to those found in the nucleated corpuscles of the *Amphiuma* and alligator; that the knots represent the remains of these resulting from or after the disturbance of the central reticulum at the time the nucleus disintegrated or was extruded, and that their density may be added to by hemoglobin retained in them.

The membrane or capsule of the erythrocyte, derived as a peripheral condensation or massing of the reticulum, must be the most resistant part, the structure last to rupture under stress. It is permeable, as is well known, allowing a ready diffusion through it. The fact that the mammalian erythrocyte assumes a spherical form before bursting, when swollen and distended by excessive endosmosis in the action of distilled water or weak acetic acid, for example, may be explained as due to its less resistant internal framework being first torn asunder and destroyed by the diffusion currents and the stress. The erythrocyte then becomes a mere turgid sac, the capsule itself rupturing at continued pressure. Both the capsule and framework are dissolved by alkalis.

The red blood corpuscle is a tissue element extremely differentiated in structure for the performance of an extremely specialized function. In the mammal it is more differentiated than in the lower vertebrates, being one of the two bodies possessed that, containing no nucleus, can no longer be called a cell. From the above studies it is concluded (1) that it normally possesses a framework in the form of a fine threaded, somewhat elastic reticulum in the meshes of which its hemoglobin is supported so intimately that its content partakes of the physical characters of

gelatin; (2) that in the nucleated forms, this same reticulum is continuous into the nucleus, supporting its structures; (3) that it possesses a peripheral membrane or capsule into which the threads of the reticulum grade and which is derived from and consists of a peripheral condensation or massing of the reticulum; (4) that there is a similar but thinner perinuclear condensation of the reticulum bounding the confines of the nucleus and forming a nuclear membrane of the nucleated forms, and (5) that the central knots, or "nucleus of the mammalian corpuscle," are masses of the material of the nuclear membrane and reticulum originally existing in the central part and result from or after the disturbance of the interior produced by the disintegration or extrusion of the nucleus.

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